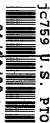


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UTILITY PATENT APPLICATION TRANSMITTAL

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Attorney Docket No.	1101-226	Total Pages	151
First Named Inventor or Application Identifier			
Vernon L. Alvarez			
Express Mail Label No.	EM 061 024 120 US		

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APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

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Submit an original, and a duplicate for fee processing)
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-Cross Reference to Related Applications
-Statement Regarding Fed sponsored R&D
-Reference to Microfiche Appendix
-Background of the Invention
-Brief Summary of the Invention
-Brief Description of the Drawings (if filed)
-Detailed Description of the Invention (including drawings, if filed)
-Claim(s)
-Abstract of the Disclosure
- ☒ Drawing(s) (35 USC 113) [Total Sheets 9]
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- ☒ Incorporation By Reference (useable if Box 4b is checked)
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: ☒ Application of: Alvarez
☐ Patent of:

☒ Serial No.: 08/488,161
☐ Patent No.:

Group Art Unit:

☒ Filed: June 7, 1995
☐ Issued:

Examiner:

For: ANTIGEN BINDING PEPTIDES (ABTIDES) Attorney Docket No.: 1101-176
FROM PEPTIDE LIBRARIES

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
[37 CFR 1.9(f) and 1.27(c)] - Small Business Concern

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I hereby declare that I am

- ☐ the owner of the small business concern identified below:
☒ an official of the small business concern empowered to act in behalf of
the concern identified below:
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Address of concern 600 College Road East
Princeton, NJ 08540

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the person employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern and/or there is an obligation under contract or law by the inventor(s) to convey rights to the small business concern with regard to the invention, entitled "ANTIGEN BINDING PEPTIDES (ABTIDES) FROM PEPTIDE LIBRARIES" by inventor(s) Alvarez described in

- ☐ the specification filed herewith
☒ application serial no. 08/488,161 filed June 7, 1995.
☐ patent no. issued

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below and no

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I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. [37 CFR 1.28 (b)]

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ANTIGEN BINDING PEPTIDES (ABTIDES)
FROM PEPTIDE LIBRARIES

TABLE OF CONTENTS

5		<u>Page</u>
	1. FIELD OF INVENTION	1
	2. BACKGROUND OF INVENTION	1
	2.1. PEPTIDE LIBRARIES	1
10	2.2. LIGANDS USED TO SCREEN PEPTIDE LIBRARIES	6
	3. SUMMARY OF THE INVENTION	9
	4. FIGURE LEGENDS	10
	5. DETAILED DESCRIPTION OF THE INVENTION	13
15	5.1. PEPTIDE LIBRARIES FOR USE IN IDENTIFYING ABTIDES	14
	5.1.1. CHEMICALLY SYNTHESIZED PEPTIDE LIBRARIES	15
20	5.1.2. BIOLOGICAL PEPTIDE LIBRARIES	16
	5.2. ABTIDES	22
	5.3. SCREENING OF PEPTIDE LIBRARIES TO IDENTIFY ABTIDES	24
25	5.3.1. ANTIBODIES AND DERIVATIVES THEREOF FOR USE IN SCREENING	35
	5.4. IDENTIFICATION OF SYNTHETIC SEQUENCES WHICH MEDIATE BINDING	37
	5.5. USES OF ABTIDES	39
30	5.5.1. ASSAYS USING ABTIDES	39
	5.5.1.1. SANDWICH ELISA	40
	5.5.2. PHARMACEUTICAL COMPOSITIONS	42
	5.5.3. IN VIVO DIAGNOSTIC AND THERAPEUTIC USES OF ABTIDES	45
35	5.6. SYNTHESIS OF PEPTIDES	48
	5.6.1. PROCEDURE FOR SOLID PHASE SYNTHESIS	48
	5.6.2. CLEAVAGE AND DEPROTECTION	50
	5.6.3. PURIFICATION OF THE PEPTIDES	50

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		<u>Page</u>
	5.6.4. CONJUGATION OF PEPTIDES TO OTHER MOLECULES	51
	5.6.4.1. BIOTINYLATION OF PEPTIDES	52
5	6. EXAMPLES	53
	6.1. ABTIDES MIMICKING THE BINDING SPECIFICITY OF MONOCLONAL ANTIBODY 7E11-C5	53
10	6.1.1. IDENTIFICATION AND ISOLATION OF ABTIDES MIMICKING THE BINDING SPECIFICITY OF MONOCLONAL ANTIBODY 7E11-C5	53
	6.1.2. CHARACTERIZATION OF 7E11-C5 ABTIDES	60
15	6.1.2.1. DOT BLOTS USING 7E11-C5 ABTIDES	60
	6.1.2.2. 7E11-C5 ABTIDES IN PLACE OF ANTIBODIES IN IMMUNOASSAYS	61
20	6.1.2.3. BIOTINYLATION OF ANTIBODIES	63
	6.1.3. 7E11-C5 ABTIDES AND MONOCLONAL ANTIBODY 7E11-C5 RECOGNIZE AN LNCaP ANTIGEN	63
	6.1.4. USE OF 7E11-C5 ANTIBODIES IN BIODISTRIBUTION STUDIES	65
25	6.2. ABTIDES BINDING TO A BREAST CANCER ANTIGEN	70
	6.2.1. IDENTIFICATION AND ISOLATION OF ABTIDES BINDING TO POLYMORPHIC EPITHELIAL MUCIN (PEM)	70
30	6.2.2. SATURATION MUTAGENESIS OF MP-1 AND IDENTIFICATION OF ADDITIONAL PEM BINDING ABTIDES	75
35		

ANTIGEN BINDING PEPTIDES (ABTIDES)
FROM PEPTIDE LIBRARIES

5 This application is a continuation-in-part of co-
pending U.S. Patent Application Serial No. 08/310,192 filed
September 21, 1994, the entire contents of which are
incorporated herein by reference.

1. FIELD OF THE INVENTION

10 The present invention relates generally to peptides
capable of specific binding to ligands of interest. The
present invention also relates to peptides capable of
mimicking the specific binding of a receptor to its ligand, an
antibody to its antigen, and the like. Such peptides are
15 known as "abtides." Abtides are identified by first and
second screening steps of peptide libraries. The first
screening step uses an antibody or receptor as a first target
ligand and identifies peptide sequences ("mimetopes") which
specifically bind to the antibody or receptor. The mimetopes
20 are then incorporated into a second target ligand in a second
screening step to identify abtides that bind the mimetope.
Abtides mimic the binding specificity of the antibody (to its
antigen) or the receptor (to its ligand) that was used as the
first target ligand in the first screening step. The
25 invention further relates to the use of abtides in the place
of antibodies in assays. The invention also provides abtide
compositions for use in therapy and diagnosis of disease.

2. BACKGROUND OF THE INVENTION

2.1. PEPTIDE LIBRARIES

30 The use of peptide libraries is well known in the
art. Such peptide libraries have generally been constructed
by one of two approaches. According to one approach, peptides
35 have been chemically synthesized *in vitro* in several formats.
For example, Fodor et al., 1991, Science 251: 767-773,
describes use of complex instrumentation, photochemistry and

computerized inventory control to synthesize a known array of short peptides on an individual microscopic slide. Houghten et al., 1991, Nature 354: 84-86, describes mixtures of free hexapeptides in which the first and second residues in each peptide were individually and specifically defined. Lam et al., 1991, Nature 354: 82-84, describes a "one bead, one peptide" approach in which a solid phase split synthesis scheme produced a library of peptides in which each bead in the collection had immobilized thereon a single, random sequence of amino acid residues. For the most part, the chemical synthetic systems have been directed to generation of arrays of short length peptides, generally fewer than about 10 amino acids or so, more particularly about 6-8 amino acids. Direct amino acid sequencing, alone or in combination with complex record keeping of the peptide synthesis schemes, is required to use these libraries.

According to a second approach using recombinant DNA techniques, peptides have been expressed in biological systems as either soluble fusion proteins or viral capsid fusion proteins.

A number of peptide libraries according to the second approach have used the M13 phage. M13 is a filamentous bacteriophage that has been a workhorse in molecular biology laboratories for the past 20 years. M13 viral particles consist of six different capsid proteins and one copy of the viral genome, as a single-stranded circular DNA molecule. Once the M13 DNA has been introduced into a host cell such as *E. coli*, it is converted into double-stranded, circular DNA. The viral DNA carries a second origin of replication that is used to generate the single-stranded DNA found in the viral particles. During viral morphogenesis, there is an ordered assembly of the single-stranded DNA and the viral proteins, and the viral particles are extruded from cells in a process much like secretion. The M13 virus is neither lysogenic nor lytic like other bacteriophage (e.g., λ); cells, once infected, chronically release virus. This feature leads to high titers of virus in infected cultures, i.e., 10^{12} pfu/ml.

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The genome of the M13 phage is ~8000 nucleotides in length and has been completely sequenced. The viral capsid protein, protein III (pIII) is responsible for infection of bacteria. In *E. coli*, the pillin protein encoded by the

5 F factor interacts with pIII protein and is responsible for phage uptake. Hence, all *E. coli* hosts for M13 virus are considered male because they carry the F factor. Several investigators have determined from mutational analysis that the 406 amino acid long pIII capsid protein has two domains.

10 The C-terminus anchors the protein to the viral coat, while portions of the N-terminus of pIII are essential for interaction with the *E. coli* pillin protein (Crissman and Smith, 1984, Virology 132: 445-455). Although the N-terminus of the pIII protein has been shown to be necessary for viral

15 infection, the extreme N-terminus of the mature protein does tolerate alterations. In 1985, George Smith published experiments reporting the use of the pIII protein of bacteriophage M13 as an experimental system for expressing a heterologous protein on the viral coat surface (Smith, 1985,

20 Science 228: 1315-1317). It was later recognized, independently by two groups, that the M13 phage pIII gene display system could be a useful one for mapping antibody epitopes. De la Cruz et al., 1988, J. Biol. Chem. 263: 4318-4322 cloned and expressed segments of the cDNA encoding the

25 *Plasmodium falciparum* surface coat protein into the pIII gene, and recombinant phage were tested for immunoreactivity with a polyclonal antibody. Parmley and Smith, 1988, Gene 73: 305-318 cloned and expressed segments of the *E. coli* β -galactosidase gene in the pIII gene and identified

30 recombinants carrying the epitope of an anti- β -galactosidase monoclonal antibody. The latter authors also described a process termed "biopanning", in which mixtures of recombinant phage were incubated with biotinylated monoclonal antibodies, and phage-antibody complexes could be specifically recovered

35 with streptavidin-coated plastic plates.

In 1989, Parmley and Smith, 1989, Adv. Exp. Med. Biol. 251:215-218 suggested that short, synthetic DNA segments

Other investigators have used other viral capsid proteins for expression of non-viral DNA on the surface of phage particles. The protein pVIII is a major M13 viral capsid protein and interacts with the single stranded DNA of 5 M13 viral particles at its C-terminus. It is 50 amino acids long and exists in approximately 2,700 copies per particle. The N-terminus of the protein is exposed and will tolerate insertions, although large inserts have been reported to disrupt the assembly of pVIII fusion proteins into viral 10 particles (Cesareni, 1992, FEBS Lett. 307:66-70). To minimize the negative effect of pVIII fusion proteins, a phagemid system has been utilized. Bacterial cells carrying the phagemid are infected with helper phage and secrete viral particles that have a mixture of both wild-type and pVIII 15 fusion capsid molecules. pVIII has also served as a site for expressing peptides on the surface of M13 viral particles. Four and six amino acid sequences corresponding to different segments of the *Plasmodium falciparum* major surface antigen have been cloned and expressed in the comparable gene of the 20 filamentous bacteriophage fd (Greenwood et al., 1991, J. Mol. Biol. 220:821-827).

Lenstra, 1992, J. Immunol. Meth. 152:149-157 described construction of a library by a laborious process encompassing annealing oligonucleotides of about 17 or 23 25 degenerate bases with an 8 nucleotide long palindromic sequence at their 3' ends. This resulted in the expression of random hexa- or octa-peptides as fusion proteins with the β -galactosidase protein in a bacterial expression vector. The DNA was then converted into a double-stranded form with Klenow 30 DNA polymerase, blunt-end ligated into a vector, and then released as Hind III fragments. These fragments were then cloned into an expression vector at the C-terminus of a truncated β -galactosidase to generate 10^7 recombinants. Colonies were then lysed, blotted on nitrocellulose filters 35 (10⁶/filter) and screened for immunoreactivity with several different monoclonal antibodies. A number of clones were isolated by repeated rounds of screening and were sequenced.

Cull et al., 1992, Proc. Natl. Acad. Sci. USA
89:1865-1869 described a system in which random peptides were
fused to the carboxy terminus of the *lac* repressor. The
fusion proteins contained an intact *lac* amino terminus (which
5 is responsible for specific binding of the *lac* repressor to
the DNA sequences constituting the *lac* operator sites). The
nucleotide sequences encoding the fusion protein were cloned
into a plasmid containing copies of the *lac* operator site.
Thus, when the fusion protein was expressed in bacteria, it
10 became bound to the operator sites of the plasmid encoding it.
This provided a physical linkage between the fusion protein
and the gene encoding it. When bacteria containing the
plasmid were screened with ligands for which it was desired to
isolate binding partners, the fusion proteins comprising
15 peptides that specifically bound to the ligand were isolated,
carrying along the genes that encoded those fusion proteins.

A comprehensive review of various types of peptide
libraries can be found in Gallop et al., 1994, J. Med. Chem.
37:1233-1251.

20

2.2. LIGANDS USED TO SCREEN PEPTIDE LIBRARIES

Screening of peptide libraries has generally been
confined to the use of a restricted number of ligands. Most
commonly, the ligand has been an antibody (Parmley and Smith,
25 1989, Adv. Exp. Med. Biol. 251:215-218; Scott and Smith, 1990,
Science 249:386-390). In many cases, the aim of the screening
is to identify peptides from the library that mimic the
epitopes to which the antibodies are directed. Thus, given an
available antibody, peptide libraries are excellent sources
30 for identifying epitopes or epitope-like molecules of that
antibody (Yayon et al., 1993, Proc. Natl. Acad. Sci. USA
90:10643-10647).

While previous studies have succeeded in identifying
epitopes and epitope-like molecules from peptide libraries, it
35 has not been realized in the prior art that this approach
could be extended by using the identified epitopes in a

further round of screening of a peptide library to identify antibody-like molecules.

When it has been desired to obtain antibody-like molecules, the prior art has employed peptide libraries that contain naturally occurring antibody sequences. This has probably been due to the fact that specific binding by antibodies is known to depend upon a complex structure involving various complementarity determining regions (CDRs), often from both heavy and light antibody chains. Short peptides would not be expected to mimic such structures and longer peptides were thought to be unsuitable for display in the most commonly used libraries.

McCafferty et al., 1990, Nature 348:552-554 used PCR to amplify immunoglobulin variable (V) region genes and cloned those genes into phage expression vectors. The authors suggested that phage libraries of V, diversity (D), and joining (J) regions could be screened with antigen. The phage that bound to antigen could then be mutated in the antigen-binding loops of the antibody genes and rescreened. The process could be repeated several times, ultimately giving rise to phage which bind the antigen strongly.

Marks et al., 1991, J. Mol. Biol. 222:581-597 also used PCR to amplify immunoglobulin variable (V) region genes and cloned those genes into phage expression vectors.

Kang et al., 1991, Proc. Natl. Acad. Sci. USA 88:4363-4366 created a phagemid vector that could be used to express the V and constant (C) regions of the heavy and light chains of an antibody specific for an antigen. The heavy and light chain V-C regions were engineered to combine in the periplasm to produce an antibody-like molecule with a functional antigen binding site. Infection of cells harboring this phagemid with helper phage resulted in the incorporation of the antibody-like molecule on the surface of phage that carried the phagemid DNA. This allowed for identification and enrichment of these phage by screening with the antigen. It was suggested that the enriched phage could be subject to mutation and further rounds of screening, leading to the

isolation of antibody-like molecules that were capable of even stronger binding to the antigen.

Hoogenboom et al., 1991, Nucleic Acids Res. 19:4133-4137 suggested that naive antibody genes might be cloned into phage display libraries. This would be followed by random mutation of the cloned antibody genes to generate high affinity variants.

In the prior art, peptide libraries have been screened with receptors to identify receptor ligand-like peptides, but peptide libraries have not been considered 10 useful for identifying such ligand-binding peptides as those that mimic receptors.

Bass et al., 1990, Proteins: Struct. Func. Genet. 8:309-314 fused human growth hormone (hGH) to the carboxy terminus of the gene III protein of phage fd. This fusion protein was built into a phagemid vector. When cells carrying the phagemid were infected with a helper phage, about 10% of the phage particles produced displayed the fusion protein on their surfaces. These phage particles were enriched by screening with hGH receptor-coated beads. It was suggested that this system could be used to develop mutants of hGH with altered receptor binding characteristics.

Lowman et al., 1991, Biochemistry 30:10832-10838 used an improved version of the system of Bass et al. 25 described above to select for mutant hGH proteins with exceptionally high affinity for the hGH receptor. The authors randomly mutagenized the hGH-pIII fusion proteins at sites near the vicinity of 12 amino acids of hGH that had previously been identified as being important in receptor binding.

30 Balass et al., 1993, Proc. Natl. Acad. Sci. USA
90:10638-10642 used a phage display library to isolate linear
peptides that mimicked a conformationally dependent epitope of
the nicotinic acetylcholine receptor. This was done by
screening the library with a monoclonal antibody specific for
35 the conformationally dependent epitope. The monoclonal
antibody used was thought to be specific to the acetylcholine

receptor's binding site for its natural ligand, acetylcholine.

Citation or identification of any reference herein shall not be construed as an admission that such reference is available as prior art to the present invention.

3. SUMMARY OF THE INVENTION

The present invention relates to abtides. As used herein, the term "abtides" refers to peptides that mimic the binding specificity of a larger molecule such as an antibody or receptor. Abtides specifically bind to a ligand of interest, in which the ligand is a specific binding partner of the larger molecule (e.g. antibody or receptor). To identify the abtides of the present invention, peptide libraries are screened in a two-step process. The first screening step uses an antibody (or antigen-binding derivative thereof) or receptor (or ligand-binding derivative thereof) as a first target ligand. This step identifies peptide sequences termed "epitopes" or "mimetopes" which specifically bind the first target ligand. In the case where an antibody or derivative thereof is used as the first target ligand, a mimetope will often resemble, either functionally in terms of its binding capability and/or structurally in terms of its amino acid sequence, the epitope recognized by the antibody used as the first ligand. An epitope or mimetope is then used as a second target ligand in a second screening step to identify a peptide sequence that specifically binds the epitope or mimetope. Such peptides are known as "abtides." Surprisingly, it was found by the current inventors and is demonstrated herein that abtides possess binding specificities strikingly similar to those possessed by the first target ligands (usually antibodies or receptors) described above.

Abtides are useful since they mimic the binding specificities of antibodies or receptors. Thus, they may be used in many instances where antibodies or receptors may be used. The present invention further relates to the use of abtides in the place of antibodies in assays such as the many

types of immunoassays known in the art. Abtides may take the place of antibodies in such assays as, for example, enzyme-linked immunosorbent assays (ELISAs) or sandwich immunoassays. The invention also provides abtide compositions for use in 5 therapy and diagnosis. In a specific example, abtides have been discovered and demonstrated to be useful in place of antibodies in enzyme-linked immunosorbent assays and in in vivo localization to prostate carcinoma in a xenograft model.

- 10 The use of abtides has many potential advantages over the use of antibodies or receptors: the smaller size of abtides allows their easier production at lower cost, reduced immunogenicity, and may facilitate their in vivo delivery if such is desired; biological reactions and functions mediated 15 by constant domains of antibodies, and cross-linking of antibodies/receptors and resulting biological effects can be avoided if desired.

4. FIGURE LEGENDS

- 20 The present invention may be understood more fully by reference to the following detailed description of the invention, examples of specific embodiments of the invention and the appended figures in which:

Figure 1 shows in schematic diagram a general method 25 for identifying an abtide by a two-step screening process. See Section 5.3 for a discussion of this method.

Figure 2 shows the binding of biotinylated monoclonal antibody 7E11-C5 to immobilized mimotope peptide 7E11-9.5. See Section 6.1.2.2 for details.

- 30 Figure 3 shows similarities in the amino acid sequences of the CDR2L and CDR3L regions of monoclonal antibody 7E11-C5 and the 7E11-C5 abtides of Table 2. The number of amino acids in the abtides that are similar to the CDRs is indicated in parentheses, along with the percent 35 homology. Dashes indicate gaps which have been added to improve the homology. In the case of clones 13 and 16, the homology with CDR2L was greatest if the sequence of CDR2L was

reversed. The sequence shown for clone 14 is SEQ ID NO: 1; the sequence shown for clone 17 is SEQ ID NO: 2; The sequence shown for clone 15 is SEQ ID NO: 3; the sequence shown for clone 13 is SEQ ID NO: 4; the sequence shown for clone 16 is 5 SEQ ID NO: 5; the sequence shown for CDR3L is SEQ ID NO: 6; the sequence shown for CDR2L is SEQ ID NO: 7; the sequence shown for CDR2L(rev) is SEQ ID NO: 8.

Figure 4 shows binding of abtides to the 7E11-9.5 mimotope peptide in a dot blot assay as described in Section 10 6.1.2.1. Numbers along the left side of the figure refer to the 7E11-C5 abtide that was spotted in the indicated position. The number 351 refers to the monoclonal antibody 7E11-C5, used as a positive control. The numbers along the top of the figure refer to the various dilutions of the abtide or the 15 monoclonal antibody that were used.

Figure 5 shows the binding of biotinylated mimetopes to immobilized abtides. □ represents binding of mimotope peptide Biotin-LYANPGMYSLHSPA-NH₂ to 7E11-C5 abtide clone 14; O represents binding of mimotope peptide Biotin- 20 LYANPGMYSLHSPA-NH₂ to 7E11-C5 abtide clone 17; ◇ represents binding of mimotope peptide Biotin-GMYSLH-NH₂ to 7E11-C5 abtide clone 14; ▲ represents binding of mimotope peptide Biotin-GMYSLH-NH₂ to 7E11-C5 abtide clone 17. See Section 6.1.2.2 for details.

25 Figure 6 shows the capture of an antigen from a lysate of LNCaP tumor cells by the monoclonal antibody 7E11-C5 and the 7E11-C5 abtide clone 14. See Section 6.1.3 for details.

Figure 7 shows the biodistribution of abtide clone 30 14-DPTA-¹¹¹In in SCID mice bearing human prostate carcinoma LNCaP xenograft tumors 2 hours (□), bar on the left for each pair of bars) or 4 hours (▤), bar on the right for each pair of bars) post-injection of 2 μg of peptide, specific activity 32 μCi/μg. See Section 6.1.4 for details.

35 Figure 8 shows the biodistribution of abtide clone 17-DPTA-¹¹¹-In in four SCID mice bearing human prostate LNCaP carcinoma xenograft tumors 2 hours (□), leftmost bar for




each group of four bars, mouse 1; , second bar from left for each group of four bars, mouse 6) or five hours (, third bar from left for each group of four bars, mouse 2; , rightmost bar for each group of four bars, mouse 4) 5 post-injection of 0.02 μg of peptide, specific activity 2.4 $\mu\text{Ci}/\text{ng}$. See Section 6.1.4 for details.






Figure 9 shows the biodistribution of ^{111}In labeled control irrelevant peptide in SCID mice bearing human prostate carcinoma LNCaP xenograft tumors 2 hours (, leftmost bar for each group of five bars; , second bar from left for each group of five bars) or 5 hours (, third bar from left for each group of five bars; , fourth bar from left for each group of five bars; , rightmost bar for each group of five bars) post-injection of 1.5 μg of peptide, specific 15 activity 30 $\mu\text{Ci}/\mu\text{g}$. See Section 6.1.4 for details.

Figure 10 schematically illustrates the construction of the R26 TSAR library. The R26 expression library was constructed essentially as described for the TSAR-9 library that is described in PCT publication WO 94/18318, dated August 20 18, 1994, except for the modifications depicted in Figure 10. The oligonucleotide assembly process depicted in Figure 10 results in expression of peptides with the following amino acid sequence:

$\text{S}(\text{S}/\text{R})\text{X}_{12}\pi\text{A}\delta\text{X}_{12}\text{SR}$ (SEQ ID NO: 89), where $\pi = \text{S}, \text{P}, \text{T}$ or A ; and 25 $\delta = \text{V}, \text{A}, \text{D}, \text{E}$ OR G .

Figure 11 schematically illustrates the construction of the D38 TSAR library. The D38 expression library was constructed essentially as described for the TSAR-9 library that is described in PCT publication WO 94/18318, dated August 30 18, 1994, except for the modifications depicted in Figure 11.

Figure 12 schematically illustrates the construction of the DC43 TSAR library. The DC43 expression library was constructed essentially as described for the TSAR-9 library that is described in PCT publication WO 94/18318, dated August 35 18, 1994, except for the modifications depicted in Figure 12.

Figure 13 schematically illustrates the oligonucleotides used to construct the polymorphic epithelial

CS434879-01800

mucin (PEM) abtide saturation mutagenesis TSAR library (See Section 6.2.2).

5. DETAILED DESCRIPTION OF THE INVENTION

5 The present invention relates generally to abtides. As used herein, the term "abtides" refers to peptides that mimic the binding specificity of a larger molecule such as an antibody or receptor. Abtides specifically bind to a ligand of interest, in which the ligand is a specific binding partner
10 of the mimicked larger molecule (e.g. antibody or receptor). To identify the abtides of the present invention, peptide libraries are typically screened in a two-step process (see Figure 1). The first screening step uses an antibody (or antigen-binding derivative thereof) or receptor (or ligand-
15 binding derivative thereof) as a first target ligand. This step identifies peptide sequences termed "epitopes" or "mimetopes" which specifically bind the first target ligand. If the first screening step uses an antibody and the peptide identified contains the amino acid sequence of the natural
20 antigen that is responsible for the specific binding of the antigen to the antibody, then the identified peptide is said to be an epitope; if the identified peptide does not contain the sequence of the natural antigen, then the identified peptide is said to be a mimetope. In the case where an
25 antibody or derivative thereof is used as the first target ligand, a mimetope will often resemble, either functionally in terms of its binding capability and/or structurally in terms of its amino acid sequence, the epitope recognized by the antibody used as the first ligand.
30 A mimetope is then used as a second target ligand in a second screening step to identify a peptide sequence that specifically binds the epitope or mimetope. Such peptides are known as "abtides." Abtides possess binding specificities similar to those possessed by the first target ligands
35 (usually antibodies or receptors) described above.

 In a specific embodiment, the antibody or derivative thereof used in the first screening step recognizes a tumor

antigen, preferably a human tumor antigen, most preferably of a malignant tumor.

The present invention provides a method to successfully screen against very small peptide or protein targets, e.g. 5 to 40 amino acids, preferably 10 to 20 amino acids. To date, screening against such targets has not been successful. The methods of the present invention increase the likelihood that the abtide obtained will bind its target in a complex or structurally dependent fashion.

10 Abtides are useful since they mimic the binding specificities of antibodies or receptors. Thus, they may be used in many instances where antibodies or receptors may be used. The present invention further relates to the use of abtides in the place of antibodies in assays such as the many
15 types of immunoassays known in the art. Abtides may take the place of antibodies in such assays as, for example, enzyme-linked immunosorbent assays (ELISAs) or sandwich immunoassays. The invention also provides abtide compositions for use in therapy and diagnosis of disease. In a specific example,
20 abtides have been produced and demonstrated to be useful in place of antibodies in enzyme-linked immunosorbent assays and in *in vivo* localization to prostate carcinoma in xenograft models.

The use of abtides has many potential advantages
25 over the use of antibodies or receptors: the smaller size of abtides allows their easier production at lower cost, reduced immunogenicity, and facilitates their *in vivo* delivery if such is desired; biological reactions and functions mediated by constant domains of antibodies, and cross-linking of
30 antibodies/receptors and resulting biological effects can be avoided if desired.

5.1. PEPTIDE LIBRARIES FOR USE IN IDENTIFYING ABTIDES

The abtides of the present invention can be
35 identified from a chemically synthesized peptide library or a biologically expressed peptide library. If a biological peptide expression library is used, the nucleic acid which

encodes the peptide which binds to the ligand of choice can be recovered, and then sequenced to determine its nucleotide sequence and hence deduce the amino acid sequence that mediates binding. Alternatively, the amino acid sequence of an appropriate binding domain can be determined by direct determination of the amino acid sequence of a peptide selected from a peptide library containing chemically synthesized peptides. In a less preferred aspect, direct amino acid sequencing of a binding peptide selected from a biological peptide expression library can also be performed.

In a preferred embodiment of the present invention, the aptamers are advantageously identified from random peptide libraries. Typically, random peptide libraries will be encoded by synthetic oligonucleotides with a plurality of variant nucleotide positions having the potential to encode all 20 naturally occurring amino acids. The sequence of amino acids encoded by the variant nucleotides is unpredictable and substantially random. The terms "unpredicted", "unpredictable" and "substantially random" are used interchangeably with respect to the amino acids encoded and are intended to mean that the variant nucleotides at any given position are such that it cannot be predicted which of the 20 naturally occurring amino acids will appear at that position. These variant nucleotides are the product of random chemical synthesis. The biological random peptide libraries envisioned for use include those in which a bias has been introduced into the random sequence, e.g., to disfavor stop codon usage.

5.1.1. CHEMICALLY SYNTHESIZED PEPTIDE LIBRARIES

The peptide libraries used in the present invention may be libraries that are chemically synthesized *in vitro*. Examples of such libraries are given in Fodor et al., 1991, Science 251:767-773, which describes the synthesis of a known array of short peptides on an individual microscopic slide; Houghten et al., 1991, Nature 354:84-86, which describes mixtures of free hexapeptides in which the first and second residues in each peptide were individually and specifically

defined. Lam et al., 1991, Nature 354:82-84, which describes a split synthesis scheme; Medynski, 1994, Bio/Technology 12:709-710, describes split synthesis and T-bag synthesis methods as well. See also Gallop et al., 1994, J. Medicinal
5 Chemistry 37:1233-1251.

PCT publication WO 91/05058, dated April 18, 1991, is directed to random libraries containing semi-random nucleotide sequences. The semi-random nucleotide sequences are transcribed in vitro under conditions such that polysomes
10 are produced. The polysomes are screened for binding to a substance of interest. Those polysomes that bind to the substance of interest are recovered. The RNA from those polysomes is used to construct cDNA, which is expressed to produce polypeptides.

15 Screening to identify peptides which bind to a ligand of choice can be carried out by methods well known in the art.

In a specific embodiment, the total number of unpredictable amino acids in the peptides of the chemical
20 library used for screening is greater than or equal to 5 and less than or equal to 25; in other embodiments the total is in the range of 5-15 or 5-10 amino acids, preferably contiguous amino acids.

While a binding domain can be identified from
25 chemically synthesized peptide libraries, such a domain would be small (i.e. less than 10 amino acids, and most probably 5-6 amino acids, in length). Therefore, use of a chemically synthesized peptide library is less preferred for the second screening step involved in isolating aptides than is use in
30 the second screening step of biological peptide libraries containing unpredictable sequences of greater length, described below.

5.1.2. BIOLOGICAL PEPTIDE LIBRARIES

35 In another embodiment, biological peptide libraries are used to identify aptides. Many suitable biological peptide libraries are known in the art and can be used.

According to this second approach, involving recombinant DNA techniques, peptides have been expressed in biological systems as either soluble fusion proteins or viral capsid fusion proteins:

- 5 A number of peptide libraries according to this approach have used the M13 phage. Although the N-terminus of the viral capsid protein, protein III (pIII), has been shown to be necessary for viral infection, the extreme N-terminus of the mature protein does tolerate alterations such as
- 10 insertions. Accordingly, various peptide libraries, in which the diverse peptides are expressed as pIII fusion proteins, are known in the art; these libraries can be used to identify abtides. Examples of such libraries are described below.
- 15 Scott and Smith, 1990, Science 249:386-390 describe construction and expression of an "epitope library" of hexapeptides on the surface of M13. The library was made by inserting a 33 base pair Bgl⁻I digested oligonucleotide sequence into an Sfi I digested phage fd-tet, i.e., fUSE5 RF.
- 20 The 33 base pair fragment contains a random or "degenerate" coding sequence (NNK), where N represents G, A, T or C and K represents G or T. The authors stated that the library consisted of 2×10^8 recombinants expressing 4×10^7 different hexapeptides; theoretically, this library expressed 69% of the
- 25 6.4×10^7 possible peptides (20%). Cwirla et al., 1990, Proc. Natl. Acad. Sci. USA 87: 6378-6382 also described a somewhat similar library of hexapeptides expressed as pIII gene fusions of M13 fd phage. PCT publication WO 91/19818 dated December 26, 1991 by Dower and Cwirla describes a similar library of
- 30 pentameric to octameric random amino acid sequences.
- Devlin et al., 1990, Science, 249:404-406, describes a peptide library of about 15 residues generated using an (NNS) coding scheme for oligonucleotide synthesis in which S is G or C.
- 35 Christian and colleagues have described a phage display library, expressing decapeptides (Christian et al., 1992, J. Mol. Biol. 227:711-718). The starting DNA was

generated by means of an oligonucleotide comprising the degenerate codons [NN(G/T)]₁₀ with a self-complementary 3' terminus. This sequence, in forming a hairpin, creates a self-priming replication site which could be used by T4 DNA polymerase to generate the complementary strand. The double-stranded DNA was cleaved at the SfiI sites at the 5' terminus and hairpin for cloning into the fUSE5 vector described by Scott and Smith, *supra*.

Lenstra, 1992, J. Immunol. Meth. 152:149-157 describes construction of a library by a laborious process encompassing annealing oligonucleotides of about 17 or 23 degenerate bases with an 8 nucleotide long palindromic sequence at their 3' ends. This resulted in the expression of random hexa- or octa-peptides as fusion proteins with the β -galactosidase protein in a bacterial expression vector. The DNA was then converted into a double-stranded form with Klenow DNA polymerase, blunt-end ligated into a vector, and then released as Hind III fragments. These fragments were then cloned into an expression vector at the sequence encoding the C-terminus of a truncated β -galactosidase to generate 10⁷ recombinants.

Other biological peptide libraries which can be used include those described in U.S. Patent No. 5,270,170 dated December 14, 1993 and PCT Publication No. WO 91/19818 dated December 26, 1991. Also suitable are those in U.S. Patent No. 5,096,815, U.S. Patent No. 5,198,346, and U.S. Patent No. 5,223,409, all to Ladner et al.

The biological peptide libraries discussed above are meant to be illustrative and not limiting. It will be recognized by one of skill in the art that many other biological peptide libraries disclosed in various publications may be suitable for use in the practice of the present invention.

The protein pVIII is a major M13 viral capsid protein, which can also serve as a site for expressing peptides on the surface of M13 viral particles, in the construction of random peptide libraries.

While it would be understood by one skilled in the art that as few as 5 amino acids can constitute a binding domain, the average functional domain within a natural protein is considered to be about 40 amino acids. Thus, the random peptide libraries from which the abtides of the present invention are preferably identified encode peptides having in the range of 5 to 200 total variant amino acids. Although it is contemplated that biologically expressed random peptide libraries displaying short random inserts (i.e. less than 20 amino acids in length) could be used to identify abtides of the invention, the most preferred biologically expressed random peptide libraries for use in the invention are those in which the displayed peptide has 20 or greater unpredictable amino acids i.e. preferably in the range of 20 to 100, and most preferably 20 to 50 amino acids, as exemplified by the TSAR libraries described in PCT publication WO 91/12328, dated August 22, 1991, and PCT publication WO 94/18318, dated August 18, 1994.

To identify abtides, particularly in the second screening step, the invention preferably uses libraries of greater complexity than are commonly employed in the art. The conventional teaching in the random peptide library art is that the length of inserted oligonucleotides should be kept short, encoding preferably fewer than 15 and most preferably about 6-8 amino acids. However, not only can libraries encoding more than about 20 amino acids be constructed, but such libraries can be advantageously screened to identify peptides having binding specificity for a variety of ligands. Such libraries with longer length inserts are exemplified by the TSAR libraries, described in PCT publication WO 91/12328, dated August 22, 1991, and PCT publication WO 94/18318, dated August 18, 1994.

These PCT publications disclose that the use of libraries composed of longer length oligonucleotides has many advantages.

Libraries composed of longer length oligonucleotides afford the ability to identify peptides in which a short

sequence of amino acids is common to or shared by a number of peptides binding a given ligand, i.e., library members having shared binding motifs. The use of longer length libraries also affords the ability to identify peptides which do not have any shared sequences with other peptides but which nevertheless have binding specificity for the same ligand.

When screened by the method of the present invention, libraries having large inserted oligonucleotide sequences provide the opportunity to identify or map binding sites which encompass not only a few contiguous amino acid residues, i.e., simple binding sites, but also those which encompass discontinuous amino acids, i.e., complex binding sites, and may afford the complex binding characteristic of antibodies and receptor-like molecules.

- 15 Additionally, the large size of the inserted synthesized oligonucleotides of certain libraries provides the opportunity for the development of secondary and/or tertiary structure in the potential binding peptides and in sequences flanking the actual binding site in the binding domain.
- 20 Secondary and tertiary structure often significantly affect the ability of a sequence to mediate binding, as well as the strength and specificity of any binding which occurs. Such complex structural effects are not possible when only small length oligonucleotides are used in libraries. It may be that
- 25 secondary and tertiary structures are especially important in the identification of aptamers since aptamers mimic the binding of large molecules such as antibodies. It is well known that the antigen binding properties of antibodies depend in most instances upon several different regions of the heavy chain
- 30 (complementarity determining regions) and upon regions contributed by the light chain as well.

Therefore, it is contemplated that the most preferred binding domains for identifying the aptamers of the present invention will be those from biologically expressed

35 random peptide libraries in which the displayed peptide is 20 or greater amino acids in length. Examples of such random peptide libraries are the TSAR libraries, described in in PCT

publication WO 91/12328, dated August 22, 1991, and PCT publication WO 94/18318, dated August 18, 1994.

In one embodiment, the library utilized in the present invention is a linear, non-constrained library. As 5 would be understood by one in the art having considered the present disclosure, in another specific embodiment, "constrained", "structured" or "semi-rigid" random peptide libraries could also be used in the present methods to identify abtides. Typically, these libraries express peptides 10 that are substantially random but contain a small percentage of fixed residues within or flanking the random sequences that have the result of conferring structure or some degree of conformational rigidity to the peptide. In a semirigid peptide library, the plurality of synthetic oligonucleotides 15 express peptides that are each able to adopt only one or a small number of different conformations that are constrained by the positioning of codons encoding certain structure conferring amino acids in or flanking the synthesized variant or unpredicted oligonucleotides. Unlike linear, unconstrained 20 libraries in which the plurality of proteins expressed potentially adopt thousands of short-lived different conformations, in a semirigid peptide library, the plurality of proteins expressed can adopt only a single or a small number of conformations. Such libraries are exemplified by the 25 TSAR-13 and TSAR-14 libraries described in PCT publication WO 94/18318, dated August 18, 1994; by a library of random 6 amino acid sequences, each flanked by invariant cysteine residues (O'Neil et al., 1992, Proteins 14:509-515); and by those libraries disclosed in PCT Publication No. WO 94/11496, 30 dated May 26, 1994 (Huse).

In a preferred embodiment, a biological peptide library that is a random peptide "TSAR" library is screened to identify an abtide. TSARs is an acronym for "Totally Synthetic Affinity Reagents" as described in PCT publication 35 WO 91/12328, dated August 22, 1991, and PCT publication WO 94/18318, dated August 18, 1994. TSAR libraries, their construction and use, and specific examples of TSAR libraries,

are described in detail in those PCT publications. Nucleic acids encoding TSARs or a TSAR portion which mediates binding to the ligand used for screening can be used to identify the abtides of the present invention.

- 5 A TSAR may be a heterofunctional fusion protein, said fusion protein comprising (a) a binding domain encoded by an oligonucleotide comprising unpredictable nucleotides in which the unpredictable nucleotides are arranged in one or more contiguous sequences, wherein the total number of
- 10 unpredictable nucleotides is greater than or equal to about 60 and less than or equal to about 600, and optionally, (b) an effector domain encoded by an oligonucleotide sequence which is a protein or peptide that enhances expression or detection of the binding domain.
- 15 Alternatively, a TSAR may be a heterofunctional fusion protein as described above but in which the contiguous sequences are flanked by invariant residues designed to encode amino acids that confer a desired structure to the binding domain of the expressed heterofunctional fusion protein.
- 20 In addition to TSAR libraries, other libraries for use in the present invention may be those wherein the library is a library of recombinant vectors that express a plurality of heterofunctional fusion proteins, said fusion proteins comprising a binding domain encoded by an oligonucleotide
- 25 comprising unpredictable nucleotides in which the unpredictable nucleotides are arranged in one or more contiguous sequences, wherein the total number of unpredictable nucleotides is greater than or equal to about 15 and less than or equal to about 600.

30

5.2. ABTIDES

- An abtide is typically a peptide that mimics, with respect to binding specificity, and possibly other
- 35 characteristics (e.g., binding affinity, sequence, etc.) a large molecule such as an antibody or receptor. However, an

described by way of example in Section 5.6 and its subsections.

Alternatively, abtides (or portions thereof) can be obtained by recombinant expression of a nucleic acid encoding the desired abtide in an appropriate host, by well-known methods.

Occasionally, it may happen that not all of the amino acids in the identified peptide will be necessary for the binding function of an abtide. Where it is desired to decrease the size of the abtide, methods can be used to identify portions of the determined synthetic amino acid or nucleotide sequences which respectively mediate binding, or encode the sequences which mediate binding, as described in Section 5.4 below.

15

5.3. SCREENING OF PEPTIDE LIBRARIES TO IDENTIFY ABTIDES

The process of identifying abtides from a peptide library comprises two distinct screening steps. The first step is designed to identify epitopes or ligands with binding specificity for the larger molecule of interest, e.g. antigen mimics, receptor-ligand mimics, and the like. In that first step, a peptide library is screened with a ligand that possesses a specific, often complex, binding site of interest. Those peptides in the library that are specific binding partners of the ligand bind to the ligand and are readily recoverable because of this specific binding. An example of a ligand suitable for use in this first screening step would be an antibody, inherently possessing an antigen binding site, or an antigen-binding derivative thereof. Another example would be a receptor e.g. the epidermal growth factor receptor or the platelet derived growth factor receptor. These particular receptors possess specific binding sites for epidermal growth factor and platelet derived growth factor, respectively. Other receptors are known in the art and are also potential ligands, for example, the estrogen receptor, the various

acetylcholine receptors, the human growth hormone receptor, etc.

Molecules comprising those peptide sequences in the library that are identified by the first screening step will be referred to herein as epitopes or mimetopes. For example, in the case where the first ligand is an antibody, the peptides in the library that are identified as specific binding partners of the antibody would be known as antigen epitopes or mimetopes.

The peptides that are isolated in the first screening step are then preferably analyzed, as, for example, by DNA sequencing of the binding domain of the phage that encode the peptides if the library used was a phage library. The DNA sequence of the binding domain encodes the amino acid sequence of the epitope or mimetope peptide. Due to the known relationship between DNA sequences and their encoded amino acid sequences, obtaining the DNA sequence of the epitope or mimetope allows the determination of the amino acid sequence of the epitope or mimetope. Alternatively, if the library used was a chemical library, direct amino acid sequencing of the peptide epitope or mimetope can be carried out by well known methods in the art.

In a specific embodiment, sequences of different peptide mimetopes identified in the first screening step can be compared to determine a consensus mimetope sequence:

Once the amino acid sequence of the epitope or mimetope is known (or a portion thereof, which mediates binding), a molecule, preferably a peptide, is produced comprising that amino acid sequence which mediates binding. This peptide may be synthesized chemically, or, alternatively, may be produced by methods involving recombinant DNA. This peptide may contain only the amino acids of the epitope or mimetope or, preferably, it may contain additional amino acids or non-amino acid moieties to aid in identifying or recovering the epitope or mimetope peptide and any new peptide binders found in the subsequent screening step discussed below.

CS-404879-011000

In the second screening step, the epitope or mimotope that was identified in the first screening step is used as a ligand for the second screening step. The second screening step identifies peptides with binding specificity for the epitope or mimotope and that surprisingly mimic the binding specificity of the antibody or receptor that was used as ligand in the first screening step. In other words, the second screening step yields peptides with antibody or receptor-like binding activity for antigens or receptor ligands that are known as abtides.

Figure 1 is a schematic representation of an exemplary two-step screening process used to identify abtides.

In a particular embodiment of the invention, it may be that the epitope to which an antibody specifically binds is known. For example, the monoclonal antibody SM-3 that specifically binds the polymorphic epithelial mucin (PEM) found on human breast carcinoma cells has been shown to be specific for the epitope defined by the amino acid sequence VTSAPDTRPAPGSTAPPAHGVTAPDTR (SEQ ID NO: 9) (Burchell et al., 1989, Int. J. Cancer 44:691-696). In such cases, the first screening step described above may be dispensed with. A peptide comprising the sequence of the epitope for which the antibody is specific can be synthesized and used in the second screening step described above in order to identify abtides of the antibody.

It may also be that the portion of a "receptor-ligand" (i.e., a ligand which specifically binds to a receptor) to which a receptor specifically binds is known. For example, it has been shown that granulocyte/macrophage colony stimulating factor (GM-CSF) binds to the GM-CSF receptor through amino acids 88-121 (HCPPTPETSCATQTITFESFKENLKDFLLVIPFDC [SEQ ID NO: 22]) of GM-CSF. It should be possible to synthesize a peptide corresponding to the portion of the receptor-ligand that has been shown to be responsible for specific binding to the receptor and to use such a peptide in the second screening

step of the methods of the present invention in order to identify an abtide of the receptor.

As used in the present invention, a ligand is a substance for which it is desired to isolate a specific binding partner from a peptide library. A ligand can function as a lock, i.e., a large polypeptide or protein analogous to a lock into which a smaller specific binding partner fits as a key; or a ligand can function as a key which fits into and specifically binds a larger binding partner or lock.

10 In this invention, an epitope or mimetope is typically a peptide that acts as a key; it is identified by screening a peptide library for peptides that fit into and bind the specific binding site of a larger molecule which acts as a lock, e.g. antibody or receptor. If the larger molecule
15 is an antibody and the peptide identified contains the portion of the amino acid sequence of the natural antigen that is responsible for the specific binding of the antigen to the antibody, then the identified peptide is said to be an epitope; if the identified peptide does not contain the
20 sequence of the natural antigen, then the identified peptide is said to be a mimetope.

In a specific embodiment, a mimetope is identified by screening a peptide library with an antibody or antibody fragment. Mimetopes thus identified functionally mimic the
25 antigen to which the antibody binds in that the mimetopes also specifically bind with the antibody. In some cases, if the antigen is a protein or polypeptide, the mimetopes may share amino acid sequence motifs with the antigen. In another embodiment, a mimetope is identified by screening a peptide
30 library with a receptor or receptor fragment. Mimetopes thus identified functionally mimic the natural ligand of the receptor.

The peptide libraries that are used in the first and second screening steps may be the same or different. In one
35 embodiment, a peptide library containing small random inserts (from about 6 to about 15 amino acids) is used in the first screening step.

In the second screening step, it may be desirable to use a larger peptide library. Such larger libraries preferably express peptides of about 20 to 200 random amino acids. Examples of such larger libraries are the TSAR 5 libraries described in PCT publication WO 91/12328, dated August 22, 1991, and in PCT Publication WO 94/18318, dated August 18, 1994.

Biological or chemically synthesized peptide libraries can be used in either the first or second 10 screenings. The peptide libraries used in the present invention may have a plurality of residues that are random, i.e. residues for which the amino acid occupying that residue cannot be predicted in advance. Such libraries are said to be random peptide libraries.

15 A preferred method for identifying abtides comprises screening a library of recombinant vectors that express a plurality of heterofunctional fusion proteins, said fusion proteins comprising (a) a binding domain encoded by an oligonucleotide comprising unpredictable nucleotides in which 20 the unpredictable nucleotides are arranged in one or more contiguous sequences, wherein the total number of unpredictable nucleotides is greater than or equal to about 15 and less than or equal to about 600, and optionally, (b) an effector domain encoded by an oligonucleotide sequence which 25 is a protein or peptide that enhances expression or detection of the binding domain. Screening is done by contacting the plurality of heterofunctional fusion proteins with a ligand under conditions conducive to ligand binding and then isolating the fusion proteins which bind to the ligand. The 30 methods of the invention further preferably comprise determining the nucleotide sequence encoding the binding domain of the heterofunctional fusion protein identified to determine the DNA sequence that encodes the binding domain and simultaneously to deduce the amino acid sequence of the 35 mimotope used in the second screen. Nucleotide sequence analysis can be carried out by any method known in the art, including but not limited to the method of Maxam and Gilbert

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(1980, Meth. Enzymol. 65:499-560), the Sanger dideoxy method (Sanger et al., 1977, Proc. Natl. Acad. Sci. U.S.A. 74:5463), the use of T7 DNA polymerase (Tabor and Richardson, U.S. Patent No. 4,795,699; Sequenase™, U.S. Biochemical Corp.), or 5 Taq polymerase, or use of an automated DNA sequenator (e.g., Applied Biosystems, Foster City, CA).

Alternatively, the libraries used to screen for mimetopes and abtides of the invention have unpredictable nucleotides arranged in one or more contiguous sequences that 10 are flanked by invariant residues designed to encode amino acids that confer a desired structure to the binding domain of the expressed heterofunctional fusion protein.

Once a suitable peptide library has been constructed (or otherwise obtained), the library is screened to identify 15 peptides having binding affinity for a ligand of choice. Screening the libraries can be accomplished by any of a variety of methods known to those of skill in the art. See, e.g., the following references, which disclose screening of peptide libraries: Parmley and Smith, 1989, Adv. Exp. Med. 20 Biol. 251:215-218; Scott and Smith, 1990, Science 249:386-390; Fowlkes et al., 1992, BioTechniques 13:422-427; Oldenburg et al., 1992, Proc. Natl. Acad. Sci. USA 89:5393-5397; Yu et al., 1994, Cell 76:933-945; Staudt et al., 1988, Science 241:577-580; Bock et al., 1992, Nature 355:564-566; Tuerk et al., 25 1992, Proc. Natl. Acad. Sci. USA 89:6988-6992; Ellington et al., 1992, Nature 355:850-852; U.S. Patent No. 5,096,815, U.S. Patent No. 5,223,409, and U.S. Patent No. 5,198,346, all to Ladner et al.; and Rebar and Pabo, 1993, Science 263:671-673. See also PCT publication WO 94/18318, dated August 18, 30 1994.

If the libraries are expressed as fusion proteins with a cell surface molecule, then screening is advantageously achieved by contacting the vectors with an immobilized target ligand and harvesting those vectors that bind to said ligand. 35 Such useful screening methods, designated "panning" techniques are described in Fowlkes et al., 1992, BioTechniques 13:422-427. In panning methods useful to screen the libraries, the

target ligand can be immobilized on plates, beads, such as magnetic beads, sepharose, etc., or on beads used in columns. In particular embodiments, the immobilized target ligand can be "tagged", e.g., using such as biotin, 2-fluorochrome, e.g. 5 for FACS sorting.

In one embodiment, presented by way of example but not limitation, screening a library of phage expressing random peptides on phage and phagemid vectors can be achieved by using magnetic beads as described in PCT publication WO 10 94/18318, dated August 18, 1994.

Alternatively, as yet another non-limiting example, screening a library of phage expressing random peptides can be achieved by panning using microtiter plates. In a preferred method for recovering the phage bound to the wells of the 15 microtiter plates, a pH change is used.

By way of another example, the libraries expressing random peptides as a surface protein of either a vector or a host cell, e.g., phage or bacterial cell, can be screened by passing a solution of the library over a column of a ligand 20 immobilized to a solid matrix, such as sepharose, silica, etc., and recovering those phage that bind to the column after extensive washing and elution.

By way of yet another example, weak binding library members can be isolated based on retarded chromatographic 25 properties. According to one mode of this embodiment for screening, fractions are collected as they come off the column, saving the trailing fractions (i.e., those members that are retarded in mobility relative to the peak fraction are saved). These members are then concentrated and passed 30 over the column a second time, again saving the retarded fractions. Through successive rounds of chromatography, it is possible to isolate those that have some affinity, albeit weak, to the immobilized ligand. These library members are retarded in their mobility because of the millions of possible 35 ligand interactions as the member passes down the column. In addition, this methodology selects those members that have

modest affinity to the target, and which also have a rapid dissociation time.

If desired, the oligonucleotides encoding the binding domain selected in this manner can be mutagenized, expressed and rechromatographed (or screened by another method) to discover improved binding activity. In particular, saturation mutagenesis can be carried out using synthetic oligonucleotides synthesized from "doped" nucleotide reservoirs. The doping is carried out such that the original peptide sequence is represented only once in 10^6 unique clones of the mutagenized oligonucleotide. The assembled oligonucleotides are cloned into a parental TSAR vector. Preferably, the vector is m663 (Fowlkes et al., 1992, BioTechniques 13:422-427). m663 is able to make blue plaques when grown in *E. coli* stain JM101 or DH5 α F'. A library of greater than 10^6 is preferred; however a library of 10^5 is sufficient to isolate TSAR phage displaying peptide domains with increased selectivity for binding to the target ligand.

According to another alternative method, screening a library of can be achieved using a method comprising an "enrichment" step and a filter lift step as follows.

Random peptides from an expressed library capable of binding to a given ligand ("positives") are initially enriched by one or two cycles of panning or affinity chromatography, as described above. The goal is to enrich the positives to a frequency of about $> 1/10^5$. Following enrichment, a filter lift assay is conducted. For example, approximately $1-2 \times 10^5$ phage, enriched for binders, are added to 500 μ l of log phase *E. coli* and plated on a large LB-agarose plate with 0.7% agarose in broth. The agarose is allowed to solidify, and a nitrocellulose filter (e.g., 0.45 μ) is placed on the agarose surface. A series of registration marks is made with a sterile needle to allow re-alignment of the filter and plate following development as described below. Phage plaques are allowed to develop by overnight incubation at 37 °C (the presence of the filter does not inhibit this process). The filter is then removed from the plate with phage from each

individual plaque adhered *in situ*. The filter is then exposed to a solution of BSA or other blocking agent for 1-2 hours to prevent non-specific binding of the ligand (or "probe").

The probe itself is labeled, for example, either by
5 biotinylation (using commercial NHS-biotin) or direct enzyme labeling, e.g., with horse radish peroxidase (HRP) or alkaline phosphatase. Probes labeled in this manner are indefinitely stable and can be re-used several times. The blocked filter is exposed to a solution of probe for several hours to allow
10 the probe to bind *in situ* to any phage on the filter displaying a peptide with significant affinity to the probe. The filter is then washed to remove unbound probe, and then developed by exposure to enzyme substrate solution (in the case of directly labeled probe) or further exposed to a
15 solution of enzyme-labeled avidin (in the case of biotinylated probe). In a preferred method, an HRP-labeled probe is detected by ECL western blotting methods (Amersham, Arlington Heights, IL), which involves using luminol in the presence of phenol to yield enhanced chemiluminescence detectable by brief
20 exposure of film by autoradiography, in which the exposed areas of film correspond to positive plaques on the original plate. Where an enzyme substrate is used, positive phage plaques are identified by localized deposition of colored enzymatic cleavage product on the filter which corresponds to
25 plaques on the original plate. The developed filter or film, as the case may be, is simply realigned with the plate using the registration marks, and the "positive" plaques are cored from the agarose to recover the phage. Because of the high density of plaques on the original plate, it is usually
30 impossible to isolate a single plaque from the plate on the first pass. Accordingly, phage recovered from the initial core are re-plated at low density and the process is repeated to allow isolation of individual plaques and hence single clones of phage.

35 Successful screening experiments are optimally conducted using 3 rounds of serial screening. The recovered cells are then plated at a low density to yield isolated

colonies for individual analysis. The individual colonies are selected and used to inoculate LB culture medium containing ampicillin. After overnight culture at 37°C, the cultures are then spun down by centrifugation. Individual cell aliquots
5 are then retested for binding to the target ligand attached to the beads. Binding to other beads, having attached thereto a non-relevant ligand, can be used as a negative control.

One important aspect of screening the libraries is that of elution. For clarity of explanation, the following is
10 discussed in terms of TSAR expression by phage; however, it is readily understood that such discussion is applicable to any system where the random peptide is expressed on a surface fusion molecule. It is conceivable that the conditions that disrupt the peptide-target interactions during recovery of the
15 phage are specific for every given peptide sequence from a plurality of proteins expressed on phage. For example, certain interactions may be disrupted by acid pH's but not by basic pH's, and *vice versa*. Thus, it may be desirable to test a variety of elution conditions (including but not limited to
20 pH 2-3, pH 12-13, excess target in competition, detergents, mild protein denaturants, urea, varying temperature, light, presence or absence of metal ions, chelators, etc.) and compare the primary structures of the TSAR proteins expressed on the phage recovered for each set of conditions to determine
25 the appropriate elution conditions for each ligand/TSAR combination. Some of these elution conditions may be incompatible with phage infection because they are bactericidal and will need to be removed by dialysis (*i.e.*, dialysis bag, Centricon/Amicon microconcentrators).

30 The ability of different expressed proteins to be eluted under different conditions may not only be due to the denaturation of the specific peptide region involved in binding to the target but also may be due to conformational changes in the flanking regions. These flanking sequences may
35 also be denatured in combination with the actual binding sequence; these flanking regions may also change their secondary or tertiary structure in response to exposure to the

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elution conditions (i.e., pH 2-3, pH 12-13, excess target in competition, detergents, mild protein denaturants, urea, heat, cold, light, metal ions, chelators, etc.) which in turn leads to the conformational deformation of the peptide responsible for binding to the target.

According to another alternative method in which the TSARs contain a linker region between the binding domain and the effector domain, particular TSAR libraries can be prepared and screened by: (1) engineering a vector, preferably a phage vector, so that a DNA sequence encodes a segment of Factor Xa (or Factor Xa protease cleavable peptide) and is present adjacent to the gene encoding the effector domain, e.g., the pIII coat protein gene; (2) construct and assemble the double stranded synthetic oligonucleotides as described above and insert into the engineered vector; (3) express the plurality of vectors in a suitable host to form a library of vectors; (4) screen for binding to an immobilized ligand; (5) wash away excess phage; and (6) treat the immobilized phage with Factor Xa protease. The particle will be uncoupled from the peptide-ligand complex and can then be used to infect bacteria to regenerate the particle with its full-length pIII molecule for additional rounds of screening. This alternative embodiment advantageously allows the use of universally effective elution conditions and thus allows identification of phage expressing TSARs that otherwise might not be recovered using other known methods for elution. To illustrate, using this embodiment, exceptionally tight binding TSARs could be recovered. If desired, the oligonucleotides encoding the binding domain selected in this manner can be mutagenized, expressed and rechromatographed (or screened by another method) to discover improved binding activity. In particular, saturation mutagenesis can be carried out using synthetic oligonucleotides synthesized from "doped" nucleotide reservoirs. The doping is carried out such that the original peptide sequence is represented only once in 10^6 unique clones of the mutagenized oligonucleotide. The assembled oligonucleotides are cloned into a parental TSAR vector.

Preferably, the vector is m663 (Fowlkes et al., 1992, BioTechniques 13:422-427). m663 is able to make blue plaques when grown in *E. coli* strain JM101 or DH5 α F'. A library of greater than 10^6 is preferred; however a library of 10^5 is sufficient to isolate TSAR phage displaying peptide domains with increased selectivity for binding to the target ligand.

For the examples in Section 6 herein, a TSAR library is utilized; however, to those skilled in the art, it will be apparent that other peptide libraries may be used. An example of a TSAR library is the TSAR-9 library disclosed in Kay et al., 1993, Gene 128:59-65. TSAR-9 constructs display a peptide of about 38 amino acids in length having 36 totally random positions.

15 5.3.1. ANTIBODIES AND DERIVATIVES THEREOF
 FOR USE IN SCREENING

Antibodies can be produced which recognize an antigen of interest. Such antibodies can be polyclonal or monoclonal. Such antibodies may be used as ligands in the first screening step of the present invention.

Various procedures known in the art may be used for the production of polyclonal antibodies to an antigen of interest. For the production of antibody, various host animals can be immunized by injection with an antigen of interest or derivative thereof, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

35 A monoclonal antibody to an antigen of interest can be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in

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culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein (1975, Nature 256:495-497), and the more recent human B cell hybridoma technique (Kozbor et al., 1983, Immunology Today 5 4:72) and EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

The monoclonal antibodies may be human monoclonal antibodies or chimeric human-mouse (or other species) 10 monoclonal antibodies. Human monoclonal antibodies may be made by any of numerous techniques known in the art (e.g., Teng et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:7308-7312; Kozbor et al., 1983, Immunology Today 4:72-79; Olsson et al., 1982, Meth. Enzymol. 92:3-16). Chimeric antibody molecules 15 may be prepared containing a mouse antigen-binding domain with human constant regions (Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851, Takeda et al., 1985, Nature 314:452).

A molecular clone of an antibody to an antigen of 20 interest can be prepared by known techniques. Recombinant DNA methodology (see e.g., Maniatis et al., 1982, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York) may be used to construct nucleic acid sequences which encode a monoclonal antibody molecule, or 25 antigen binding region thereof.

Antibody molecules may be purified by known techniques, e.g., immunoabsorption or immunoaffinity chromatography, chromatographic methods such as HPLC (high performance liquid chromatography), or a combination thereof, etc.

30 Antibody fragments which contain the idiotype or antigen binding region of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')₂ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments 35 which can be generated by reducing the disulfide bridges of the F(ab')₂ fragment, and the 2 Fab or Fab fragments which can

be generated by treating the antibody molecule with papain and a reducing agent.

5.4. IDENTIFICATION OF SYNTHETIC SEQUENCES WHICH MEDIATE BINDING

- 5 When a peptide from a peptide library has been identified as an abtide or mimetope for a particular target ligand of interest according to the method of the invention (in either the first or second screening step), it may be
- 10 useful to determine what region(s) of the expressed peptide sequence is (are) responsible for binding to the target ligand. Such analysis can be conducted at two different levels, i.e., the nucleotide sequence and amino acid sequence levels.
- 15 By molecular biological techniques it is possible to verify and further analyze a ligand binding peptide at the level of the oligonucleotides. First, the inserted oligonucleotides can be cleaved using appropriate restriction enzymes and religated into the original expression vector and
- 20 the expression product of such vector screened for ligand binding to identify the oligonucleotides that encode the binding region of the abtide or mimetope. Second, the oligonucleotides can be transferred into another vector, e.g., from phage to phagemid. The newly expressed fusion proteins
- 25 should acquire the same binding activity if the domain is necessary and sufficient for binding to the ligand. This last approach also assesses whether or not flanking amino acid residues encoded by the original vector influence peptide binding in any fashion. Third, the oligonucleotides can be
- 30 synthesized, based on the nucleotide sequence determined for the phage in the library that encodes the binding peptide, amplified by cloning or PCR amplification using internal and flanking primers, cleaved into two pieces and cloned as two half-binding domain fragments. In the foregoing manner, the
- 35 inserted oligonucleotides are subdivided into two equal halves. If the peptide domain important for binding is small, then one recombinant clone would demonstrate binding and the

other would not. If neither have binding, then either both are important or the essential portion of the domain spans the middle (which can be tested by expressing just the central region).

- 5 Alternatively, by synthesizing peptides corresponding to the deduced sequence of the abtide or mimetope, the binding domains can be analyzed. First, the entire peptide should be synthesized and assessed for binding to the target ligand to verify that the peptide is necessary
10 and sufficient for binding. Second, short peptide fragments, for example, overlapping 10-mers, can be synthesized, based on the amino acid sequence of the random peptide binding domain, and tested to identify those binding the ligand.

- In addition, in certain instances, linear motifs
15 (consensus motifs) may become apparent after comparing the primary structures of different binding peptides from the library having binding affinity for a target ligand. The contribution of these motifs to binding can be verified with synthesized peptides in competition experiments (i.e.,
20 determine the concentration of peptide capable of inhibiting 50% of the binding of the phage to its target; IC_{50}). Conversely, the motif or any region suspected to be important for binding can be removed from or mutated within the DNA encoding the random peptide insert and the altered displayed
25 peptide can be retested for binding.

- Furthermore, once the binding domain of a peptide has been identified, the binding characteristics of that peptide can be modified by varying the binding domain sequence to produce a related family of peptides with differing
30 properties for a specific ligand.

- Moreover, in a method of directed evolution, the identified peptides can be improved by additional rounds of random mutagenesis, selection, and amplification of the nucleotide sequences encoding the binding domains.
35 Mutagenesis can be accomplished by creating and cloning a new set of oligonucleotides that differ slightly from the parent sequence, e.g., by 1-10%. Selection and amplification are

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achieved as described above. By way of example, to verify that the isolated peptides have improved binding characteristics, mutants and the parent phage, differing in their lacZ expression, can be processed together during the screening experiments. Alteration of the original blue-white color ratios during the course of the screening experiment will serve as a visual means to assess the successful selection of enhanced binders. This process can go through numerous cycles.

10

5.5. USES OF ABTIDES

5.5.1. ASSAYS USING ABTIDES

The abtides of the present invention possess binding specificities that are similar to those of the ligands (e.g. antibodies, receptors) that are used in the first screening step of the process by which the abtides are identified. Consequently, the abtides may be used in many of the same instances where the ligand of the first screening step might be used. For example, if the ligand used in the first screening step is an antibody, the abtide that is identified after the second screening step will bind specifically to the same antigen to which the antibody specifically binds. Therefore, the abtide may be used as a substitute for the antibody in many of the reactions or assays that the antibody could be used in. For example, the abtide could be used in immunoassays known in the art, e.g., those designed to detect or measure the amount of the antigen. Of course, such immunoassays may have to be suitably modified. For example, many immunoassays make use of a step in which a second antibody, labeled with a radioactive moiety or an enzyme such as alkaline phosphatase, specifically binds to the first antibody. Such a second antibody would not be expected to specifically bind to the abtide. However, it would be well within the competence of one of ordinary skill in the art to fabricate another labelling moiety, perhaps a third antibody,

that was able to specifically bind to the abtide, or to label the abtide with a detectable marker prior to use.

- The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems
- 5 using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assays), "sandwich" immunoassays, dot immunoblot assays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays,
- 10 complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, immunoaffinity chromatography, and flow dipstick assays to name but a few. For examples of exemplary procedures which can be used in immunoassays, see generally Kricka, 1985,
- 15 Clinical and Biochemical Analysis 17:1-15; Armbruster, 1993, Clin. Chem. 39/2:181-195; Birnbaum et al., 1992, Anal. Biochem. 206:168-171; Miyai, 1985, Adv. Clin. Chem. 24:61-110; and references cited therein.

- The samples to be assayed in the immunoassays can be
- 20 any sample that may contain the antigen or ligand desired to be assayed. For example, these samples can be body fluids such as plasma, blood, serum, saliva, cerebrospinal fluid, synovial fluid, etc.

- The detectable label to be used in the immunoassays
- 25 can be any detectable label known in the art. Such labels include radioisotopes, fluorescent dyes, enzymes (for example horseradish peroxidase or alkaline phosphatase), chemiluminescent molecules, metal atoms, or phosphorescent dyes, colored particles, metal and dye colloids.

30

5.5.1.1. SANDWICH ELISA

- In a particular embodiment, the abtides can be used in a sandwich enzyme immunoassay. One description of such an embodiment, presented by way of example and not limitation,
- 35 follows: A molecule comprising an abtide is affixed to a solid substratum. The molecule comprising the abtide may be linked to a substance that will provide for greater attachment

- of the molecule to the substratum. The sample to be assayed is contacted with the molecule comprising the abtide that is bound to the substratum. The substances in the sample that are specific binding partners of the abtide (analyte) will
- 5 bind to the abtide, and non-binding sample components are removed by washing. An enzyme-conjugated monoclonal antibody directed against the analyte is added. This enzyme-conjugated monoclonal antibody binds to the part of the analyte it is specific for and completes the sandwich. After removal of
- 10 unbound enzyme-conjugated monoclonal antibody by washing, a substrate solution is added to the wells. A colored product is formed in proportion to the amount of analyte present in the sample. The reaction may be terminated by addition of stop solution and absorbance is measured
- 15 spectrophotometrically. The following illustrates these steps in more detail.
- (a) Polystyrene microtiter wells (Flow Laboratory) are coated overnight at room temperature with 100 μ l of a solution of a molecule comprising an abtide at a concentration of 1 mg/ml in phosphate buffered saline (PBS).
 - 20 (b) Coating solution is discarded and wells are blocked for 1-2 hours at room temperature with 300 μ l of 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) with 0.05% of Tween 20 (PBS-Tween buffer).
 - 25 (c) 150 μ l of sample (suspected of containing an analyte the presence or amount of which it is desired to measure) diluted in 1% BSA-PBS is added per well. Wells are incubated 1 hour at room temperature.
 - 30 (d) Wells are washed 4 times with PBS-Tween buffer.
 - (e) 100 μ l of horseradish peroxidase conjugated monoclonal antibody specific for the analyte in 1% BSA-PBS is added per well. The
 - 35 concentration of the monoclonal antibody can be

from about 10 ng/ml to 10 mg/ml. Wells are incubated 1 hour at room temperature.

- (f) Wells are washed 6 times with PBS -Tween buffer.
- 5 (g) 100 μ l of ABTS[®] Boehringer Mannheim (2,2'-Azino-di-[3-ethylbenzthiazdine sulfonate (6)] crystallized diammonium salt working solution is added per well. ABTS[®] stock solution is prepared at 15 mg/ml in dH₂O. To make the
- 10 working solution, 200 μ l of this ABTS[®] stock is diluted into 10 ml of citrate phosphate buffer (17 mM citric acid, 65 mM dibasic sodium phosphate) and 10 μ l 30% H₂O₂.
- (h) The absorbance of each well is measured at 405
- 15 nm in a microtiter plate reader (Dynatech MR600, Dynatech Corp., Alexandria, VA.).

5.5.2. PHARMACEUTICAL COMPOSITIONS

The invention provides methods of treatment by
20 administration to a subject of an effective amount of a
pharmaceutical (therapeutic or diagnostic) composition
comprising an abtide. Such an abtide envisioned for
therapeutic or diagnostic use is referred to hereinafter as a
"Therapeutic" or "Therapeutic of the invention." Such
25 therapeutics are abtides that specifically bind to a molecule
in vivo, to exert a therapeutic or diagnostic effect. In a
preferred aspect, the Therapeutic is substantially purified.
The subject is preferably an animal, including but not limited
to animals such as cows, pigs, horses, chickens, cats, dogs,
30 etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed are known in the art and can be selected from among those described hereinbelow.

Various delivery systems are known and can be used 35 to administer a Therapeutic of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells containing the Therapeutic, receptor-

mediated endocytosis (see, e.g., Wu and Wu, 1987, J. Biol. Chem. 262:4429-4432), etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, 5 epidural, and oral routes as well as transdermal and subcutaneous time-release implants. The Therapeutics may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and 10 intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, 15 including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. In a specific embodiment, it may be desirable to utilize liposomes targeted 20 via antibodies to specific identifiable cell surface antigens.

In a specific embodiment, it may be desirable to administer the Therapeutics of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during 25 surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic 30 membranes, or fibers.

The present invention provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a Therapeutic, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but 35 is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The carrier and

composition can be sterile. The formulation should suit the mode of administration.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The Therapeutics of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium,

calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the Therapeutic of the invention which will be effective in the treatment of a particular disorder or
5 condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the
10 route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more
15 of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the
20 agency of manufacture, use or sale for human administration.

5.5.3. IN VIVO DIAGNOSTIC AND THERAPEUTIC

USES OF ABTIDES

Another area where abtides can be used in place of
25 antibodies is in the imaging, detection, or treatment of disease. Current diagnostic and therapeutic methods make use of antibodies to target imaging agents or therapeutic substances, e.g. to tumors. Since abtides possess the same specificity of binding as antibodies, abtides can be used in
30 place of antibodies in such diagnostic and therapeutic methods.

Abtides may be linked to chelators such as those described in U.S. Patent No. 4,741,900 or U.S. Patent No. 5,326,856. The abtide-chelator complex may then be
35 radiolabeled to provide an imaging agent for diagnosis or treatment of disease. The abtide may also be used in the methods that are disclosed in co-pending U.S. patent

application serial no. 08/127,351 for creating a radiolabeled peptide for use in imaging or radiotherapy. This application contains a review of methods of using peptides in imaging agents.

- 5 In *in vivo* diagnostic applications, specific tissues or even specific cellular disorders may be imaged by administration of a sufficient amount of a labeled abtide of the instant invention.

A wide variety of metal ions suitable for *in vivo* tissue imaging have been tested and utilized clinically. For imaging with radioisotopes, the following characteristics are generally desirable: (a) low radiation dose to the patient; (b) high photon yield which permits a nuclear medicine procedure to be performed in a short time period; (c) ability to be produced in sufficient quantities; (d) acceptable cost; (e) simple preparation for administration; and (f) no requirement that the patient be sequestered subsequently. These characteristics generally translate into the following: (a) the radiation exposure to the most critical organ is less than 5 rad; (b) a single image can be obtained within several hours after infusion; (c) the radioisotope does not decay by emission of a particle; (d) the isotope can be readily detected; and (e) the half-life is less than four days (Lamb and Kramer, "Commercial Production of Radioisotopes for Nuclear Medicine", In Radiotracers For Medical Applications, Vol. 1, Rayudu (Ed.), CRC Press, Inc., Boca Raton, pp. 17-62). Preferably, the metal is technetium-99m.

By way of illustration, the targets that one may image include any solid neoplasm, certain organs such a lymph nodes, parathyroids, spleen and kidney, sites of inflammation or infection (e.g., macrophages at such sites), myocardial infarction or thromboses (neoantigenic determinants on fibrin or platelets), and the like evident to one of ordinary skill in the art. Furthermore, the neoplastic tissue may be present in bone, internal organs, connective tissue, or skin.

As is also apparent to one of ordinary skill in the art, one may use the present invention in *in vivo* therapeutics

about 2 to 5 hours. Current methods of tumor imaging using antibodies require approximately 24 to 48 hours.

Because abtides are peptides, they are cleared from the blood faster than antibodies. This means that there will be less background signal in the bloodstream when using abtides to image disease states than there is when using antibodies.

Peptides most likely will provoke less of an immune response in patients than do large proteins such as antibodies. This consideration is especially important when diagnosis or treatment is required to be done repeatedly or over a long period of time.

Abtides, because they are generally small proteins, can remain soluble in physiological fluids under conditions where antibodies cannot.

Abtides, again because they are generally peptides, can be produced synthetically or by recombinant methods and therefore may be less costly to produce than antibodies.

Abtides may be used individually. Alternatively, abtides may be used as compositions of abtides in which the peptide sequences of the abtides differ.

5.6. SYNTHESIS OF PEPTIDES

25 5.6.1. PROCEDURE FOR SOLID PHASE SYNTHESIS

Abtide or mimotope peptides may be prepared by methods that are known in the art. For example, in brief, solid phase peptide synthesis consists of coupling the carboxyl group of the C-terminal amino acid to a resin and successively adding N-alpha protected amino acids. The protecting groups may be any known in the art. Before each new amino acid is added to the growing chain, the protecting group of the previous amino acid added to the chain is removed. The coupling of amino acids to appropriate resins is described by Rivier et al., U.S. Patent No. 4,244,946. Such solid phase syntheses have been described, for example, by Merrifield, 1964, J. Am. Chem. Soc. 85:2149; Vale et al.,

1981, Science 213:1394-1397; Marki et al., 1981, J. Am. Chem. Soc. 103:3178 and in U.S. Patent Nos. 4,305,872 and 4,316,891. In a preferred aspect, an automated peptide synthesizer is employed.

- 5 By way of example but not limitation, peptides can be synthesized on an Applied Biosystems Inc. ("ABI") model 431A automated peptide synthesizer using the "Fastmoc" synthesis protocol supplied by ABI, which uses 2-(1H-Benzotriazol-1-yl)-1,1,3,3,-tetramethyluronium
- 10 hexafluorophosphate ("HBTU") (R. Knorr et al., 1989, Tet. Lett., 30:1927) as coupling agent. Syntheses can be carried out on 0.25 mmol of commercially available 4-(2',4'-dimethoxyphenyl)-(9-fluorenyl-methoxycarbonyl)-aminomethyl)-phenoxy polystyrene resin ("Rink
- 15 resin" from Advanced ChemTech) (H. Rink, 1987, Tet. Lett. 28:3787). Fmoc amino acids (1 mmol) are coupled according to the Fastmoc protocol. The following side chain protected Fmoc amino acid derivatives are used: FmocArg(Pmc)OH; FmocAsn(Mbh)OH; FmocAsp(^tBu)OH; FmocCys(Acm)OH;
- 20 FmocGlu(^tBu)OH; FmocGln(Mbh)OH; FmocHis(Tr)OH; FmocLys(Boc)OH; FmocSer(^tBu)OH; FmocThr(^tBu)OH; FmocTyr(^tBu)OH. [Abbreviations: Acm, acetamidomethyl; Boc, tert-butoxycarbonyl; ^tBu, tert-butyl; Fmoc, 9-fluorenylmethoxycarbonyl; Mbh, 4,4'-dimethoxybenzhydryl;
- 25 Pmc, 2,2,5,7,8-pentamethylchroman-6-sulfonyl; Tr, trityl]. Synthesis is carried out using N-methylpyrrolidone (NMP) as solvent, with HBTU dissolved in N,N-dimethylformamide (DMF). Deprotection of the Fmoc group is effected using ca. 20% piperidine in NMP. At the end of each synthesis the
- 30 amount of peptide present is assayed by ultraviolet spectroscopy. A sample of dry peptide resin (ca. 3-10 mg) is weighed, then 20% piperidine in DMA (10 mL) is added. After 30 min sonication, the UV (ultraviolet) absorbance of the dibenzofulvene-piperidine adduct (formed by cleavage of the
- 35 N-terminal Fmoc group) is recorded at 301 nm. Peptide substitution (in mmol g⁻¹) can be calculated according to the equation:

$$\text{substitution} = \frac{A \times v}{7800 \times w} \times 1000$$

where A is the absorbance at 301 nm, v is the volume of 20% piperidine in DMA (in mL), 7800 is the extinction coefficient (in $\text{mol}^{-1}\text{dm}^3\text{cm}^{-1}$) of the dibenzofulvene-piperidine adduct, and w is the weight of the peptide-resin sample (in mg).

Finally, the N-terminal Fmoc group is cleaved using 20% piperidine in DMA, then acetylated using acetic anhydride and pyridine in DMA. The peptide resin is thoroughly washed with DMA, CH_2Cl_2 and finally diethyl ether.

5.6.2. CLEAVAGE AND DEPROTECTION

By way of example but not limitation, cleavage and deprotection can be carried out as follows: The air-dried peptide resin is treated with ethylmethyl-sulfide (EtSMe), ethanedithiol (EDT), and thioanisole (PhSMe) for approximately 20 min. prior to addition of ~95% aqueous trifluoroacetic acid (TFA). A total volume of ca. 50 mL of these reagents are used per gram of peptide-resin. The following ratio is used: TFA : EtSMe : EDT : PhSMe (10 : 0.5 : 0.5 : 0.5). The mixture is stirred for 3 h at room temperature under an atmosphere of N_2 . The mixture is filtered and the resin washed with TFA (2 x 3 mL). The combined filtrate is evaporated in vacuo, and anhydrous diethyl ether added to the yellow/orange residue. The resulting white precipitate is isolated by filtration. See King et al., 1990, Int. J. Peptide Protein Res. 36:255-266 regarding various cleavage methods.

5.6.3. PURIFICATION OF THE PEPTIDES

Purification of the synthesized peptides can be carried out by standard methods including chromatography (e.g., ion exchange, affinity, and sizing column chromatography, high performance liquid chromatography (HPLC)), centrifugation, differential solubility, or by any other standard technique.

5.6.4. CONJUGATION OF PEPTIDES TO OTHER MOLECULES

The abtides of the present invention may be linked to other molecules (e.g., a detectable label, a molecule facilitating adsorption to a solid substratum, or a toxin, 5 according to various embodiments of the invention) by methods that are well known in the art. Such methods include the use of homobifunctional and heterobifunctional cross-linking molecules.

The homobifunctional molecules have at least two 10 reactive functional groups, which are the same. The reactive functional groups on a homobifunctional molecule include, for example, aldehyde groups and active ester groups. Homobifunctional molecules having aldehyde groups include, for example, glutaraldehyde and subaraldehyde. The use of 15 glutaraldehyde as a cross-linking agent was disclosed by Poznansky et al., 1984, Science 223:1304-1306.

Homobifunctional molecules having at least two active ester units include esters of dicarboxylic acids and N-hydroxysuccinimide. Some examples of such N-succinimidyl 20 esters include disuccinimidyl suberate and dithio-bis-(succinimidyl propionate), and their soluble bis-sulfonic acid and bis-sulfonate salts such as their sodium and potassium salts. These homobifunctional reagents are available from Pierce, Rockford, Illinois.

25 The heterobifunctional molecules have at least two different reactive groups. Some examples of heterobifunctional reagents containing reactive disulfide bonds include N-succinimidyl 3-(2-pyridyl-dithio)propionate (Carlsson et al., 1978, Biochem J. 173:723-737), sodium S-4- 30 succinimidylloxycarbonyl-alpha-methylbenzylthiosulfate, and 4-succinimidylloxycarbonyl-alpha-methyl-(2-pyridyldithio)toluene. N-succinimidyl 3-(2-pyridyldithio)propionate is preferred. Some examples of heterobifunctional reagents comprising reactive groups having 35 a double bond that reacts with a thiol group include succinimidyl 4-(N-maleimidomethyl)cyclohexahe-1-carboxylate and succinimidyl m-maleimidobenzoate.

Other heterobifunctional molecules include succinimidyl 3-(maleimido)propionate, sulfosuccinimidyl 4-(p-maleimido-phenyl)butyrate, sulfosuccinimidyl 4-(N-maleimidomethyl-cyclohexane)-1-carboxylate, maleimidobenzoyl-5 N-hydroxy-succinimide ester. The sodium sulfonate salt of succinimidyl m-maleimidobenzoate is preferred. Many of the above-mentioned heterobifunctional reagents and their sulfonate salts are available from Pierce.

Additional information regarding how to make and use these as well as other polyfunctional reagents may be obtained from the following publications or others available in the art:

- Carlsson et al., 1978, Biochem. J. 173:723-737.
Cumber et al., 1985, Methods in Enzymology 112:207-224.
- 15 Jue et al., 1978, Biochem 17:5399-5405.
Sun et al., 1974, Biochem. 13:2334-2340.
Blattler et al., 1985, Biochem. 24:1517-152.
Liu et al., 1979, Biochem. 18:690-697.
Youle and Neville, 1980, Proc. Natl. Acad. Sci. USA 77:5483-20 5486.
Lerner et al., 1981, Proc. Natl. Acad. Sci. USA 78:3403-3407.
Jung and Moroi, 1983, Biochem. Biophys. Acta 761:162.
Caulfield et al., 1984, Biochem. 81:7772-7776.
Staros, 1982, Biochem. 21:3950-3955.
- 25 Yoshitake et al., 1979, Eur. J. Biochem. 101:395-399.
Yoshitake et al., 1982, J. Biochem. 92:1413-1424.
Pilch and Czech, 1979, J. Biol. Chem. 254:3375-3381.
Novick et al., 1987, J. Biol. Chem. 262:8483-8487.
Lomant and Fairbanks, 1976, J. Mol. Biol. 104:243-261.
- 30 Hamada and Tsuruo, 1987, Anal. Biochem. 160:483-488.
Hashida et al., 1984, J. Applied Biochem. 6:56-63.

Additionally, methods of cross-linking are reviewed by Means and Feeney, 1990, Bioconjugate Chem. 1:2-12.

35 5.6.4.1. BIOTINYLATION OF PEPTIDES

Methods of biotinylating peptides are well known in the art. Any convenient method may be employed in the

7E11-C5 was bound to a well of a microtiter plate. 7E11-C5 at a concentration of 11.2 mg/mL in phosphate buffered saline (PBS), pH 6.0, was diluted to 100 μ g per mL in 0.1x PBS pH 7.2. One hundred microliters (100 μ L) of this dilution was added to one well of a microtiter plate, and allowed to incubate for 1-6 hours at room temperature or overnight at 4° C. After incubation, the well was washed at least 4 times with a blocking buffer which consisted of either 1% bovine serum albumin (BSA) in PBS, 1% non-fat dry milk (NFDM) in PBS, or 0.1% Tween® in either 1% BSA in PBS or 1% NFDM in PBS. Two hundred microliters of the blocking buffer was then added to the well and allowed to incubate for at least an hour at room temperature.

Next, an aliquot of the TSAR-9 library was added to the well containing bound 7E11-C5. An aliquot of the library containing 10^{10} phage particles was added to the well and allowed to incubate for at least 1 hour at room temperature. This resulted in the binding to the plate of those phage containing binding domains that bind to 7E11-C5. After an hour, the well was washed extensively with either 1% bovine serum albumin (BSA) in PBS, 1% non-fat dry milk (NFDM) in PBS, or 0.1% Tween® in either 1% BSA in PBS or 1% NFDM in PBS.

After washing, phage bound to the 7E11-C5 antibody in the well were eluted by adding 100 μ L of an acid solution of 0.2 M glycine-HCl, pH 2.0. After incubation from 15 minutes to 1 hour, the acid solution containing eluted phage was transferred to a 1.5 mL microfuge tube, and an equal volume of 0.2 M Tris-HCl, pH 7.5 added to neutralize the acid solution. In some cases, the neutralized phage solution was immediately added to a second well containing bound 7E11-C5 antibody, and the binding and elution procedure repeated.

If it was desired that the level of enrichment be monitored during the above steps, an irrelevant phage that

does not bind 7E11-C5 but that expresses the β -galactosidase gene was added to the aliquot from the TSAR-9 library. This phage gives rise to blue plaques when plated in the presence of X-Gal and IPTG. Following a screening
5 step, the eluted phage were plated in X-gal and IPTG. An aliquot of unscreened phage were plated as well. The ratio of white to blue plaques was measured for both phage samples. The increase in the proportion of white plaques (from the TSAR-9 phage that bind to 7E11-C5) to blue
10 plaques (from the irrelevant phage) indicated the degree to which the screening process enriched the population of phage for those phage that bind 7E11-C5.

If it was desired that the specificity of binding
15 be monitored during the above screening steps, screening was done against an irrelevant target (either BSA, mouse IgG, or plastic) in addition to being done against 7E11-C5. The enrichment of white plaques over blue plaques when
20 panning was done against 7E11-C5 rather than an irrelevant target indicated the level of specificity of binding.

After screening, the phage were amplified by adding an aliquot of the eluted phage to a solution containing LB broth and competent DH5 α F' *E. coli* cells (GIBCO BRL, Gaithersburg, MD). Typically a
25 2-5 μ L aliquot of the phage solution was added to 125 μ L of LB broth containing a 1:50 dilution of DH5 α F' *E. coli* cells (about 1×10^9 cells/ml). 3.3 mL of top agar was added to this solution, and the mixture was plated out onto a Petri dish containing agar. Often the phage were titered by
30 making several serial 1:10 dilutions, and plating out the dilution as described above. After incubation overnight at 37° C, the plates were evaluated for growth of plaques, and counted if desired. The plates were eluted by adding 3-5
35 mL of 100 mM NaCl, 10 mM MgCl₂, 10 mM Tris-HCl, pH 7.5 (SM buffer) to each plate and incubating for 1-5 hours with gentle rocking. The solution was then removed from the

plate, centrifuged, and either stored, amplified further, or analyzed.

In some cases, the phage were amplified in solution by adding 1-5 μ L of the phage solution to 1-5 mL of LB broth containing a 1:50 or 1:100 dilution of competent DH5 α F' *E. coli* cells (about 1×10^9 cells/ml. After incubation for either 6 hours or overnight, the solution was centrifuged and the supernatant collected. In some cases, the phage particles were precipitated with polyethylene glycol (PEG) by adding a 1/5 volume of PEG to the clarified phage solution, and incubating for 1 hour on ice. After centrifugation, the phage were usually reconstituted with 100 μ L of SM buffer.

Using the above procedures, nine different phage were isolated that expressed peptides containing binding domains that were capable of binding monoclonal antibody 7E11-C5. Molecules comprising these binding domains are thus mimetopes of the antigen recognized by the monoclonal antibody 7E11-C5. The binding domains of the peptides expressed by the nine phage were sequenced according to standard methods of DNA sequencing (Sequenase™, U.S. Biochemical Corp., Cleveland, OH). The determination of those DNA sequences allowed the determination of the amino acid sequences of these mimetopes. These sequences are shown in Table 1. Examination of these amino acid sequences showed that they shared a common motif of MYxxLH (SEQ ID NO. 10).

In order to use the mimetopes to identify abtides, peptides corresponding to the mimetope sequences were synthesized, and then dissolved in either water or PBS to give a final concentration of 5 $\mu\text{g/mL}$. Specifically, a peptide 5 called 7E11-9.5, with the sequence LYANPGMYSRLHSPA (SEQ ID NO: 20) and a peptide with the sequence GMY SRLHSPA (SEQ ID NO: 21) were synthesized.

First the mimetope peptide 7E11-9.5 was tested for its ability to bind to the monoclonal antibody 7E11-C5. 10 Ninety-six well plates (Immunlon 4, Dynatech, Alexandria, VA) were coated with 50 μL of a 5 $\mu\text{g/mL}$ solution of the mimetope peptide 7E11-9.5 and incubated overnight at room temperature. Following incubation, the wells were washed 4 15 times with 1% BSA in PBS. Biotinylated monoclonal antibody 7E11-C5 was serially diluted with PBS beginning with a concentration of 29 $\mu\text{g/mL}$ and various amounts of the monoclonal antibody were added to the wells that had been coated with the mimetope peptide. After incubating for 1 20 hour at room temperature, the wells were washed four times with 1% BSA in PBS and 2 times with PBS. Then, 100 μL of a 1:2000 dilution of Extravidin-Alkaline Phosphatase (4,250 units/mL) (Sigma, St. Louis, MO) in PBS was added to each well. After an hour, the plate was again washed 4 times 25 with PBS and 100 μL of a 1 mg/mL solution of the enzyme substrate p-nitrophenyl phosphate was added to each well. Color was allowed to develop for 15 minutes to 1 hour and absorbance was read at 405 nm. Figure 2 shows the results. It can be seen from Figure 2 that monoclonal antibody 7E11- 30 C5 binds to the synthesized mimetope 7E11-9.5 in a concentration dependent manner.

Next, the mimetope peptide was used to isolate abtides from a peptide library. Fifty to one hundred microliters of the solution of this peptide was used to 35 coat the wells of a 96-well plate, the wells were blocked with 0.5 % BSA in PBS, and the wells were used for

in Table 2 for clone 15 is SEQ ID NO: 3. The amino acid sequence shown in Table 2 for clone 13 is SEQ ID NO: 4. The amino acid sequence shown in Table 2 for clone 16 is SEQ ID NO: 5.

5 It was of interest to determine whether there might be some structural basis for the similarity in binding characteristics between the monoclonal antibody 7E11-C5 and the 7E11-C5 abtides. The amino acid sequences of the complementarity determining regions (CDRs) of the monoclonal
10 antibody 7E11-C5 were determined by sequencing cDNA clones of the genes encoding the variable regions of the antibody. These CDRs are responsible for the specific binding of monoclonal antibody 7E11-C5 to its antigen. Figure 3 presents a comparison of the amino acid sequences of the abtides of
15 Table 2 and portions of the amino acid sequences of some of the CDRs of monoclonal antibody 7E11-C5. Surprisingly, it can be seen that there are similarities in the sequences of these abtides and the sequences of the CDRs of the monoclonal antibody.

20

6.1.2. CHARACTERIZATION OF 7E11-C5 ABTIDES

7E11-C5 abtides were tested for their ability to bind to the 7E11-C5 mimetopes that were used as target ligands in the second screening step above. The DNA sequences of the
25 regions of the phage DNA encoding the abtides were determined. This allowed the determination of the amino acid sequences of the abtides. Based upon these determined amino acid sequences, synthetic peptides corresponding to these sequences were made. These synthetic peptides (7E11-C5 abtides) were 38
30 amino acids in length.

6.1.2.1. DOT BLOTS USING 7E11-C5 ABTIDES

In some cases, these abtides were used in a dot blot experiment. In those cases, 1 μ L of a 1 mg/mL solution of the
35 38-residue abtides was spotted onto nitrocellulose (0.2 μ m or 0.45 μ m, Schleicher & Schuell, Keene, NH) strips or circles. After drying (about 1/2 hour), the nitrocellulose

was blocked for 1 hour in a solution of 1% BSA in PBS. The nitrocellulose was then allowed to incubate in approximately 5 mL of a solution of 0.1 mg/mL of a biotinylated 7E11-9.5 mimotope peptide (biotin-
5 LYANPGMYSRLHSPA). This mimotope peptide was one of those described in Section 6.1.1 above that were synthesized based upon the nine peptides that were identified in the screening of Section 6.1.1 above. After an hour, the nitrocellulose was washed approximately 5 times with a
10 solution of 1% BSA in PBS. A 1:2000 dilution of Extravidin-Alkaline Phosphatase (4,250 units/mL) (Sigma, St. Louis, MO) in PBS was then added and allowed to incubate for 1 hour, after which the nitrocellulose was again washed extensively. Finally, a solution of 5-bromo-
15 4-chloro-3-indolyl phosphate (0.15 mg/mL) and nitro blue tetrazolium (0.3 mg/mL) (Sigma, St Louis, MO) (BCIP/NBT) was added as an enzyme substrate. Color was allowed to develop and the absorbance at 405 nm was read.

20 An example of such a dot blot assay is shown in Figure 4. In Figure 4, the 7E11-C5 abtides known as clone 14, clone 17, clone 15, clone 16, and clone 13 were tested for their ability to bind the biotinylated 7E11-9.5 mimotope peptide. Also tested, as a positive control, was
25 the monoclonal antibody 7E11-C5. 7E11-C5 was spotted onto the region marked 351 in Figure 4. Inspection of Figure 4 shows that at least three of the abtides (clone 14, clone 17, and clone 15) bound the mimotope. This shows that these abtides are capable of mimicking the specific binding
30 exhibited by the monoclonal antibody 7E11-C5.

6.1.2.2. 7E11-C5 ABTIDES IN PLACE OF ANTIBODIES IN IMMUNOASSAYS

35 The ability of abtides synthesized having the amino acid sequence encoded by the random inserts of the

phage that bound the 7E11-9.5 mimetope was further evaluated by ELISA assay methods.

The 7E11-C5 abtides clone 14 and clone 17 (See Table 2) were each dissolved in 0.1X PBS to give a solution of 5 $\mu\text{g/mL}$. Fifty microliters of each of these solutions was used to coat the wells of a 96-well microtiter plate (Immulon 4, Dynatech, Alexandria, VA) by overnight incubation at 4° C. Following this incubation, the abtide solutions were removed and the wells were blocked with 200 μL μl of a solution of 1% BSA in PBS. Mimetope peptides 7E11-9.5 (LYANPGMYSRLHSPA [SEQ IN NO: 20]) and GMYSLRHSPA (SEQ ID NO: 21) were biotinylated as described in Section 5.6.4.1 and dissolved in H_2O to give 1 mg/mL solutions. Serial 1:2 dilutions were made of these solutions and these dilutions were added to the wells of the microtiter plate containing the bound abtides. After incubation for 1 hour at room temperature, the wells were washed four times with 1% BSA in PBS. Then a 1:2000 dilution of Extravidin-Alkaline Phosphatase (4,250 units/mL) (Sigma, St. Louis, MO) in PBS was added to each well and incubated for 1 hour at room temperature. Following incubation, the wells were washed four times with 1% BSA in PBS and then twice in PBS. One hundred microliters of a 1 mg/mL solution of p-nitrophenyl phosphate in diethanol amine (DEA) buffer (both from Kirkegaard & Perry Laboratories, Gaithersburg, MD) was then added and, after incubation for 15-30 minutes at room temperature, the absorbance of the solutions in the wells was read at 405 nm. The results are shown in Figure 5. Figure 5 shows that, except for the non-linear effect at high concentrations of mimetope, there is a good correlation between the amount of mimetope added to the wells and the absorbance at 405 nm. The use of antibodies in assays such as enzyme-linked immunosorbent assays (ELISAs) to measure the concentration of a substance is well known in the art. The ability of antibodies to

specifically bind their antigens is crucial to the success of such assays. Since abtides also specifically bind to antigens, it was of interest to determine if abtides can be used in place of antibodies in immunoassays such as ELISA-like assays to measure the concentration of a substance. The results of Figure 5 show that the abtides of the present invention can be used in assays in much the same way that antibodies can be used in immunoassays such as ELISAs.

6.1.2.3. BIOTINYLATION OF ANTIBODIES

Methods of biotinylating antibodies are well known in the art. Any convenient method may be employed in the practice of the invention. For example, the following procedure was used:

- (1) dissolve 10 mg of antibody in 1 mL of PBS;
- (2) add 0.44 mg of biotin-LC-NHS (Pierce, Rockford, IL);
- (3) incubate for 30 minutes at room temperature;
- (4) purify over a Superose 12 column (Pharmacia, Piscataway, NJ).

6.1.3. 7E11-C5 ABTIDES AND MONOCLONAL ANTIBODY 7E11-C5 RECOGNIZE AN LNCaP ANTIGEN

The monoclonal antibody 7E11-C5 recognizes a prostate specific mucin antigen of the human prostate cancer cell line LNCaP (Horoszewicz et al., 1987, Anticancer Res. 7:927-936). To determine if 7E11-C5 abtides recognize and bind the native antigen, the following experiment was done.

A sandwich assay using the LNCaP antigen was performed. The wells of a microtiter plate were coated with either monoclonal antibody 7E11-C5, the clone 14 7E11-C5 abtide, or, as a negative control, BSA. Coating and washing was as for the assay described in Section 6.1.2.2.

One hundred microliters of a lysate of LNCaP cells was added to the wells. The LNCaP lysate was prepared as described in PCT publication WO 94/18318, dated August 18, 1994. Following capture of the lysate on the plate, 100 μ L of a 5 μ g/mL solution of biotinylated 7E11-C5 monoclonal antibody was added to each well. Following incubation and washing as in Section 6.1.2.2, a 1:2000 dilution of Extravidin-Alkaline Phosphatase (4,250 units/mL) (Sigma, St. Louis, MO) in PBS was added to each well and incubated for 1 hour at room temperature. Following incubation, the wells were washed four times with 1% BSA in PBS and then twice in PBS. One hundred microliters of a 1 mg/mL solution of p-nitrophenyl phosphate in diethanol amine (DEA) buffer (both from Kirkegaard & Perry Laboratories, Gaithersburg, MD) was then added and, after incubation at room temperature for 15-30 minutes, the absorbance of the solutions in the wells was read at 405 nm.

The results are shown in Figure 6. Figure 6 shows that the 7E11-C5 abtide is capable of recognizing the native 7E11-C5 antigen in LNCaP lysates. This was a surprising discovery and would not have been predicted by those skilled in the art. It was generally felt that screening a library with a mimetope could yield a binder to that mimetope. Whether such a binder could also bind the native epitope that the mimetope mimics was unknown. The mimetope could have represented the epitope in a loose fashion, e.g. its primary sequence could be slightly modified; its secondary structure or factors influencing the presentation of the mimetope could be different from those presenting the native epitope. In such a case, the binder to the mimetope would not be specific for the native epitope. The foregoing is presented as possible explanation and not as a limitation of the present invention.

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Abptides modified with DTPA were labeled with $^{111}\text{InCl}_2$ as follows. 0.1 to 0.5 mCi of $^{111}\text{InCl}_2$ (Amersham, Chicago, IL) were first neutralized by adding an equal volume of 0.1 M NaOAc, and then added to 100 to 200 μg of the DTPA-A-modified abtide. After incubation for one half hour, the labeled peptide was purified using a Superose-12 FPLC column (Pharmacia, Piscataway, NJ) with PBS as the running buffer. Labeled fractions were collected in a fraction collector. Tubes containing the labeled peptide were pooled and used to prepare syringes for injection into mice.

In one experiment, abtide clone 14-DPTA- ^{111}In (see Table 2) was injected intravenously (i.v.) into two groups of mice bearing measurable LNCaP xenografts. About 0.2 mL of a 10 $\mu\text{g}/\text{mL}$ solution of the radioactively labeled abtide in sterile saline was used. The specific activity of the abtide was about 32 $\mu\text{Ci}/\mu\text{g}$. Thus, the total injected dose of radioactivity was about $120\text{-}140 \times 10^6$ cpm.

The first group of mice was sacrificed 2 hours after injection of the abtide and tissues were dissected for analysis. The second group was sacrificed 4 hours after injection. Dissected tissues were weighed and the amount of ^{111}In in them was determined by gamma counting. The cpm per gram of each tissue was calculated by dividing the cpm of ^{111}In found in the tissue by the weight in grams of the tissue. The data are presented as the ratio of the cpm/g in each organ to the cpm/g in blood (organ to blood ratio). This gave the ratios that are shown in Table 3 and Figure 7.

TABLE 3

BIODISTRIBUTION OF ABTIDE CLONE 14-DPTA-¹¹¹In
IN LNCaP XENOGRAFT BEARING MICE

	Tissue ^c	Group 1 ^a		Group 2 ^b	
		AVG	s.e.m.	AVG	s.e.m.
	Blood	1.00	0.00	1.00	0.00
10	Lung	0.81	0.04	1.06	0.26
	Spleen	0.83	0.16	1.42	0.74
	Liver	0.95	0.04	2.12	0.69
	Kidney-R	55.85	10.22	171.73	77.97
15	Kidney-L	53.03	11.97	182.79	86.38
	Tumor	1.85	0.80	3.91	2.85
	Muscle	0.33	0.03	0.42	0.01
	Testes-R	0.54	0.02	0.94	0.25
20	Testes-L	0.68	0.24	0.88	0.24

^a Group 1: sacrificed at 2 hours; n=2.

^b Group 2: sacrificed at 4 hours; n=2.

^c Value shown is the organ to blood ratio.

25

Table 3 and Figure 7 show that, with the exception of kidney, the highest organ to blood ratio is found in the tumor, both at 2 hours and at 4 hours post-injection of abtide. This result shows that abtides with the binding specificity of antibodies, e.g. that are specific for tumor antigens, can be used to localize to those tumors.

No unusual localization was seen to any non-tumor tissue or organ except kidney. The ratio for kidney is extremely high due to the well known tendency of injected peptides to localize to the kidneys prior to being cleared from the body.

In another experiment, abtide clone 17-DPTA-¹¹¹In (see Table 2) was injected intravenously into four SCID mice bearing measurable LNCaP xenografts. Administration of xenografts was as above. About 0.2 mL of a 0.1 µg/mL solution of the radioactively labeled clone 17 abtide in sterile saline was injected. The specific activity of the abtide was about 2.4 µCi/ng. Thus, the total injected dose of radioactivity was about 100-110 x 10⁶ cpm.

In this experiment, mice were sacrificed at either 2 or 5 hours post-injection with labeled abtide. Again, as above, the data are presented as organ to blood ratios. As shown in Table 4 and Figure 8, abtide clone 17-DPTA-¹¹¹In localized to LNCaP xenograft tumors in mice.

15

TABLE 4

BIODISTRIBUTION OF ABTIDE CLONE
17-DPTA-¹¹¹In IN LNCaP XENOGRAFT BEARING MICE

20	TISSUE ^a	GROUP 1 MOUSE #		GROUP 2 MOUSE #	
		1	6	2	3
	BLOOD	1.00	1.00	1.00	1.00
	LUNG	2.52	2.57	4.33	2.47
25	SPLEEN	5.82	3.00	5.53	3.70
	LIVER-S	5.42	5.58	8.37	4.03
	KIDNEY-R	235.63	234.88	321.09	106.74
	KIDNEY-L	563.71	220.69	424.64	104.09
30	TUMOR-S	10.60	15.01	8.36	2.90
	MUSCLE	0.93	2.96	1.16	3.04
	TESTES-R	2.71	2.19	2.31	3.15
	TESTES-L	1.64	1.14	5.36	3.41

35

^a Value shown is the organ to blood ratio.

Table 4 and Figure 8, like Table 3 and Figure 7, show that the injected abtide localized to the tumor. This indicates that abtides can be useful in the localization of tumors.

In contrast to the results of the two experiments described above, in which radioactively labeled abtides were shown to localize to tumors, when the same experiments were done with a radioactively labeled control (non-abtide) peptide (the tripeptide GYK-DPTA), no specific localization to tumors was observed. This can be seen in Figure 9, which shows the biodistribution results for experiments using the ¹¹¹In-labeled control peptide.

The radiolabeled peptide conjugate

15 GYK-DPTA-¹¹¹In was injected intravenously into 5 SCID mice bearing measurable LNCaP xenografts. Mice were dissected 2 hours (n=2) and 5 hours (n=3) after injection of 1.5 µg of control peptide having a specific activity of 30 µCi/µg. The organ to blood ratios are presented in Table 5 and Figure 9.

20 As shown, the control peptide did not selectively localize to the tumor. While the tumor to blood ratio in one mouse was 3.26, the control peptide distributed equally well to other organs (e.g. lung 3.52, spleen 3.27, liver 21.70, etc.). These results show that there was non-specific uptake of the

25 control peptide in these organs. While abtide clone 14-DPTA-¹¹¹In demonstrated a tumor to blood ratio of only 1.85 at 2 hours (which appears lower than that obtained with the control peptide), clone 14-DPTA-¹¹¹In demonstrated specific localization to the tumor as the organ to blood ratios in the

30 other organs were much lower (e.g. lung 0.81, spleen 0.83, liver 0.95, etc.).

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TABLE 5

BIODISTRIBUTION OF GYK-DPTA-¹¹¹In
IN LNCaP XENOGRAFT BEARING MICE

5

	GROUP 1		GROUP 2		
	MOUSE #		MOUSE #		
ORGAN/BLOOD	1	2	3	4	5
BLOOD	1.00	1.00	1.00	1.00	1.00
10 LUNG	2.96	3.52	0.95	2.84	2.39
SPLEEN	1.74	3.27	0.97	2.21	4.04
LIVER-S	31.79	21.70	21.56	25.38	17.78
KIDNEY-R	563.87	406.27	273.82	509.53	269.30
15 KIDNEY-L	584.69	417.98	297.65	433.97	280.50
TUMOR-S	1.14	3.26	0.86	1.68	1.87
MUSCLE	1.04	1.02	0.29	4.34	2.51
TESTES-R	603.01	58.49	0.82	2.05	2.25
20 TESTES-L	44.65	2.08	0.93	2.11	2.32

25

6.2. ABTIDES BINDING TO A BREAST CANCER ANTIGEN

6.2.1. IDENTIFICATION AND ISOLATION OF ABTIDES BINDING TO
POLYMORPHIC EPITHELIAL MUCIN (PEM)

30

The monoclonal antibody SM-3 that specifically binds the polymorphic epithelial mucin (PEM) tumor antigen found on human breast cancer cells has been shown to be specific for the epitope defined by the amino acid sequence

35

VTSAPDTRPAPGSTAPPAHGVTSPDTR (SEQ ID NO: 9) (Bruchell et al., 1989, Int. J. Cancer 44:691-696). A peptide comprising this sequence was synthesized and used to isolate abtides from TSAR

peptide libraries by methods analogous to those described above. In these experiments, the specific TSAR libraries used were R26, D38 and DC43. See Figures 10-12 for description of these libraries. Phage bound to PEM were eluted by either 5 standard acid elution methods, stringent acid elution methods where phage were incubated with the PEM peptide for only 10 minutes prior to washing and elution, or were eluted using excess PEM peptide. Phage from each library were isolated that express peptides capable of binding to PEM. The amino acid sequences of PEM binding phage are shown in Table 6.

15

20

25

30

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TABLE 6
Sequences of PEM Binding Phage

Acid Eluted
R26 Library

5
A15 SFMDYFFHTPEPKPAGYPNAYTDPKHFA (SEQ ID NO: 26)
A54 SSSIFDYAPFSWGSAGLSNSSINVFERS (SEQ ID NO: 27)
A5 SASLWDALGGWTTSAVPSYPRPHQTPGR (SEQ ID NO: 28)
A39 SLGLPWIDVFGRSSAEPWPFGRTNLPRS (SEQ ID NO: 29)
10 A16 SVHGAFLDSFFPWAAADGPHGRGRL-TSF (SEQ ID NO: 30)

DC43 Library

15
MA-8 EEKQGGRWSTMMPRPWCHEGGCGFLYYDAMTKPKTPPIMRTAA (SEQ ID
NO: 31)
MA-21 LPRPFDDASWKLRAVKESPDGCGFGSPLLFPPYSGLPTFSSCD (SEQ ID
NO: 32)
20 V22 GSFESARGVTCIGNHSGIAHGCGPLRSYASFNRGSGRRH (SEQ ID
NO: 33)

D38 Library

25
MA-32 DQIGSRPQTTSRSISGSWWENAKTLWQQDYAFSAPNAA (SEQ ID
NO: 34)
V23 LSDAWGNFTTSYRDSAGFPESHAMTTSQGGKRNHASRFP (SEQ ID
30 NO: 35)
V21 VQLDDTSPRASGQETSQSEYDARPLLKFAIPRPSR (SEQ ID NO: 36)
V1 IDSSKNRISGTGYLSFPHIRHANRRHMADDSNLAPGPS (SEQ ID
NO: 37)

35

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Table 6 (continued)

Sequences of PEM Binding Phage

Acid Eluted: "Stringent"

DC43 Library

5

V44 WSIGHTHTGPEGKFRIPCDRSGCGGTTTLTHGGLNSSPTGQHERP (SEQ ID
NO: 38)

10 V39 DPCEDGYWLSSVGRAGASIRGCGAIRRSSRTLTAEYSTRASNH (SEQ ID
NO: 39)

V10 GSKRSCWGTTISNYFRPVPEGCGSASSINPNTNTGRLPSLHRQ (SEQ ID
NO: 40)

V7 SSASSGCLGRAEHLDLDSVWGCGSQADMSRRYSPWYGRPRTGV (SEQ ID
NO: 41)

15 V4 NVMWSSSKAGIRDSCSQVPPGGCGPVNRHRASPLTPFRHGSIR (SEQ ID
NO: 42)

D38 Library

20

V45 PLTSGSSSEYRNRDDCPVYKYATNCPRLNFSPSRYSPPF (SEQ ID
NO: 43)

25 V32 GDAYGGIFSRPRQGLADSYIHASYTGKHHFRGPRPPTR (SEQ ID
NO: 44)

V27 STCIGAEGEWKSFHNLQCRDATSTSSSTLDPTALRFG (SEQ ID
NO: 45)

V40 YSATLWDQFGSRQVELWSNRHASSALPFASRASVLGSR (SEQ ID
NO: 46)

30

Peptide Eluted

R26 Library

35

P24 ILGWPFLLTGLGDSTVHPRGRKGTDP (SEQ ID NO: 47)

P49 SIPSFSMWLNQLGSAALPSKGNQDRSD (SEQ ID NO: 48)

P26 SRDDIFTGGPLVLFGRGSKTSHNDVHSMR (SEQ ID NO: 49)

P6 RAEVLNWWYEFHVTAEATPVINSHNMT (SEQ ID NO: 50)

6.2.2. SATURATION MUTAGENESIS OF MP-1 AND IDENTIFICATION

OF ADDITIONAL PEM BINDING ABTIDES

5 A saturation mutagenesis library based on one of the
PEM abtides, MP-1, was constructed. Nucleotide sequences
encoding the MP-1 abtide were synthesized using a doping scheme
similar to that described in Section 5.3 whereby each nucleotide
was contaminated with 9% of each of the other 3 nucleotides
10 (e.g. G= 73% G, 9% A, 9% T, 9% C). The resulting mutagenic
oligonucleotides were used to construct a library by TSAR
library methods described above (see Figure 13).

 The resulting library was screened to identify phage
expressing abtides capable of binding to PEM. The binding of
15 isolated phage to PEM was confirmed by an ELISA assay. Phage
that were shown to bind to PEM as well as phage that did not
bind to PEM were sequenced to determine the amino acid sequences
of the expressed abtides. Table 7 shows the amino acid
sequences of these positive and negative binding phage.

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25

30

35

Table 7

Sequence Comparison: MP-1 Binding Motif

Positive Binding Sequences

MP1	GAPVWRGNPRWRGPGGFKWPGCGNGPMCNTFTTPARGGSRNNGP (SEQ ID NO: 51)
5	
E4	VSTGWSGTPRWCAPGGKQSGCGNGPRWTTLTPLDGGTRKYGP (SEQ ID NO: 57)
E7	GAPLWCEKLSGTGSGGFKWPGCGSGPTTYNTFTPARVGS DNKWP (SEQ ID NO: 58)
E16	GGPVWSAKSRWTGTGVLNWP GCGKVPSCSTYTPSRDRSRKSDP (SEQ ID NO: 59)
E21	GSALLTSKGCVRGPGGLMRPGCGNDRLGKSSYAHGGWIKTGP (SEQ ID NO: 60)
10 E33	GSPVWSGDNRWGRSSPLKRP GCGNGAKCNTLKDNRKDSRKT KH (SEQ ID NO: 61)
E44	G PLLPGEAAVHGARGLMRSGCGNGPTWNRLTAACRDSRNKGP (SEQ ID NO: 62)
E65	GSPVWVGSTRTWTHGWFRS QGCGNVPTNSCAPAGKDSQNKGP (SEQ ID NO: 63)
E73	GAPVWRGNRWCS DNGELERPGCGYGRPNILPPGRGNSRKPS (SEQ ID NO: 64)
E84	GSSGWKVKHRCGGPGTLQRPGCGNLPLGHTFPPTRGGS HMEGA (SEQ ID NO: 65)
15 E85	GPRSWMGQPRGSDAGSCKWAGCGDAPMWRAS TFGHGGPPNRGS (SEQ ID NO: 66)
E88	EALVCRGKPPWSGPAGLLWQCGTGPVSRFT TSAQGRSRNKTS (SEQ ID NO: 67)
E90	GAPVVGDIILWCSGARGAKWPGCGKOPTNKTF SHSRGGTQKSGL (SEQ ID NO: 68)
E22	GAPVSRCKPACGGFWGVNWP GCGNASMCKTFTNGHGVSSDN GH (SEQ ID NO: 69)
E29	GAHGYKNGSTCTGLGGWRCRGCGK GAMCNPSPAGGAYHNQGP (SEQ ID NO: 70)
20 E62	G PQGSEHQCCSGHWGLKFP GCGNGPICNNFTALRGASRKN GP (SEQ ID NO: 71)
E64	GEPVWCRHSGGRVQGGLDWL GCGDGLRYTVTPARGGSPKH GP (SEQ ID NO: 72)
E66	GLSLVRGDSWGSAGGKWRHCGCHGPMYNPQT PARGGSCTRNT (SEQ ID NO: 73)
E67	VSRAWSGKPRLMGSHGLNCP GCGKGHS GIMFIDPAGSANTPP (SEQ ID NO: 74)
E68	CAPMWSGKPPWC VGGGVKFRGCGNRPD CNIITPRLVESRDKAL (SEQ ID NO: 75)
25 E70	ADPVC SRKPDGGGLRGLRWPGCGKGPILYNVTAARGGSRNNGP (SEQ ID NO: 76)

Negative Binding Sequences

MPI	GAPVWRGNPRWRGPGGFKWPGCGNGPMCNTFTTPARGGSRNNGP (SEQ ID NO: 51)
30 E3	GTRVPPGFALRGGRDGLSWAGCGKAPISKYTSARGRSRKKGS (SEQ ID NO: 77)
E15	RSAVSEGKPREIVPGGCMWPGCGNGRKSNTLTHGPEQFQEIEP (SEQ ID NO: 78)
E24	SSGVGNKGKPRSWAPDALNGGCGNIQFANTITPDRGSGC NQTL (SEQ ID NO: 79)
E27	GSSVCGGQPSGRGFGGLPGPGCGNGPTSNLTLSARGGFPNKG L (SEQ ID NO: 80)
E37	GAPLWQGDPADEVLGGSMPGCGIGALSQTFTTPTPGGSRKNVT (SEQ ID NO: 81)
35 E43	AGREL RQDEGEGGAGADVARLREGPICSTFTPARGGSCPSGL (SEQ ID NO: 82)
E49	QARVSMALSCRSGPSDLMHQGCYGP RCNPDTTDSGGSHNTPT (SEQ ID NO: 83)
E60	GDPECRGKPRGRWTGSLACTGCGNGPNSKICTRARGVSRNKGP (SEQ ID NO: 84)
E72	STPGCSGYSGSGDPRLCTTACGNGHTRKTLTPAHGRSTHKEP (SEQ ID NO: 85)
E34	GQPECRITSGCCGTGDNKWL GCGKVDMCNTLNPAVGCHGTNGS (SEQ ID NO: 86)
E83	REPVVGKGPWCRGPGGLRWRGCGKSGQDKIITLSRDNRDRKRP (SEQ ID NO: 87)

When the sequences shown in Table 7 are compared (see particularly the amino acid residues marked in boldface type), it is possible to determine the influence of particular amino acid residues at specific positions in the sequence on a 5 peptide's ability to bind to PEM. Abptides that bind to PEM can be characterized by the formula:

$R_1R_2R_3R_4R_5R_6R_7R_8R_9R_{10}R_{11}R_{12}R_{13}R_{14}R_{15}R_{16}R_{17}R_{18}R_{19}R_{20}R_{21}R_{22}R_{23}R_{24}R_{25}R_{26}R_{27}R_{28}R_{29}$
 $R_{30}R_{31}R_{32}R_{33}R_{34}R_{35}R_{36}R_{37}R_{38}R_{39}R_{40}R_{41}R_{42}R_{43}$ (SEQ ID NO: 88)

10

where:

- $R_1 = G, C, E, \text{ or } V, \text{ preferably } G;$
 $R_2 = A, S, P, \text{ or } L, \text{ preferably } A;$
15 $R_3 = P, T, H, \text{ or } L, \text{ preferably } P;$
 $R_4 = L, M, Q, G, A, \text{ or } S;$
 $R_5 = W \text{ or } Y, \text{ preferably } W;$
 $R_6 = S, C, K \text{ or } T, \text{ preferably } S;$
 $R_7 = E, S, C, D, V, \text{ or } R; \quad -$
20 $R_8 = N, H, K, S, \text{ or } E;$
 $R_9 = L, H, R, N, Q, T, \text{ or } G;$
 $R_{10} = W, P, R, T, \text{ or } D, \text{ preferably } W;$
 $R_{11} = W, C, V, L, \text{ or } G, \text{ preferably } W;$
 $R_{12} = S, T, M, \text{ or } H, \text{ preferably } S \text{ or } T;$
25 $R_{13} = G;$
 $R_{14} = S, A, G, N, Q, \text{ or } H, \text{ preferably } S;$
 $R_{15} = W, H, G, A, \text{ or } R;$
 $R_{16} = G, T, E, P, V, \text{ or } W, \text{ preferably } G;$
 $R_{17} = V, F, W, K, \text{ or } A;$
30 $R_{18} = K, Q, D, E, R, \text{ or } L, \text{ preferably } K;$
 $R_{19} = R, F, \text{ or } S, \text{ preferably } R;$
 $R_{20} = P, S, I \text{ or } H, \text{ preferably } P;$
 $R_{21} = G;$
 $R_{22} = C;$
35 $R_{23} = G;$
 $R_{24} = D, S, T, N, \text{ or } H;$
 $R_{25} = G, D, L, \text{ or } R;$
 $R_{26} = P \text{ or } S, \text{ preferably } P;$
 $R_{27} = M, S, D, I, L, \text{ or } R;$
 $R_{28} = G, W, C, L, F, Y, \text{ or } T, \text{ preferably } G \text{ or } W;$

- R_{29} = S, N, V, F, H, or R;
 R_{30} = N, A, S, M, or R, preferably N;
 R_{31} = F, Q, P, or V, preferably F;
 R_{32} = S, V, I, K, A, or S;
5 R_{33} = P, A, N, or Y, preferably P;
 R_{34} = G, N, or L;
 R_{35} = K, R, C, Q or L, preferably K or R;
 R_{36} = V, K, R, or A;
 R_{37} = G, D, A, or E, preferably G;
10 R_{38} = S, T, P, Y or W; preferably S;
 R_{39} = R, I, L, P, A or S;
 R_{40} = N, K, or M, preferably N or K;
 R_{41} = S, R, T, E, Q, P, Y or H;
 R_{42} = G, A, S, D, N, P, Y, or K, preferably G;
15 R_{43} = P, H or A.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described 20 herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures 25 of which are incorporated by reference in their entireties.

30

35

WHAT IS CLAIMED IS:

1. A molecule comprising a peptide which binds to a substance of interest, which peptide is identified by a method comprising:
- 5 (a) screening a first random peptide library with a first ligand, said first ligand being a specific binding partner of said substance of interest, to identify a first peptide that specifically binds to said first ligand; and
- 10 (b) screening a second random peptide library with a second ligand comprising said first peptide identified in step (a), to identify a second peptide which binds to said second ligand and which binds to said substance of interest.
- 15
2. A molecule comprising a peptide which binds to an antigen of interest, which peptide is identified by a method comprising:
- 20 (a) screening a first random peptide library with an antibody or antigen-binding derivative thereof that specifically binds to an antigen of interest, to identify a first peptide that specifically binds to said antibody or antigen-binding derivative thereof; and
- 25 (b) screening a second random peptide library with a compound comprising said first peptide identified in step (a), to identify a second peptide which binds to said compound and which binds to said antigen of interest.
- 30
3. The molecule of claim 2, in which said first random peptide library is a different library from said second random peptide library.
- 35

4. The molecule of claim 2, in which said first random peptide library is the same library as said second random peptide library.

5 5. The molecule of claim 1, in which said method further comprises comparing the sequences of a plurality of different first peptides identified as binding said first ligand in step (a), to identify a consensus binding sequence, in which said second ligand of step (b) comprises said consensus binding
10 sequence.

6. The molecule of claim 2, in which said method further comprises comparing the sequences of a plurality of different first peptides identified as binding said antibody or
15 antigen-binding derivative thereof in step (a), to identify a consensus binding sequence, in which said compound of step (b) comprises said consensus binding sequence.

7. The molecule of claim 1 in which the first ligand
20 comprises a receptor.

8. The molecule of claim 2 in which the antibody is the monoclonal antibody 7E11-C5.

25 9. The molecule of claim 1 in which the library of step (a) or step (b) is a library of recombinant vectors that express a plurality of heterofunctional fusion proteins, said fusion proteins comprising a binding domain encoded by an oligonucleotide comprising unpredictable nucleotides in which
30 the unpredictable nucleotides are arranged in one or more contiguous sequences, wherein the total number of unpredictable nucleotides is greater than or equal to about 15 and less than or equal to about 600.

35 10. The molecule of claim 2 in which the library of step (a) or step (b) is a library of recombinant vectors that express a plurality of heterofunctional fusion proteins, said fusion proteins comprising a binding domain encoded by an oligonucleotide comprising unpredictable nucleotides in which the unpredictable nucleotides are arranged in one or more

contiguous sequences, wherein the total number of unpredictable nucleotides is greater than or equal to about 15 and less than or equal to about 600.

- 5 11. The molecule of claim 1 in which the library of step (a) or step (b) is a chemically synthesized library.

12. A molecule comprising: an amino acid sequence
10 selected from the group consisting of:

GIINANDPLPFFWFMSPYTPGPAPIDINASRALVSNESG (SEQ ID NO: 1),
CGRAYCLSGNYNIFGALFPGVSTPYADVGHDDAQSWRR (SEQ ID NO: 3),
DLSRNLDGFRFLLYNAYVPGFTPTFISLTAEHLSSPKG (SEQ ID NO: 2),
RCSPIWGISYPFGLLSSNPGVCHSSDAETNIRNDILTT (SEQ ID NO: 4),

- 15 and
GHSNYCFVSTLGMPIVGFPFSINARGLIHYGGSDPRLAA (SEQ ID NO: 5);
or a binding portion thereof.

13. A peptide in which the amino acid sequence of
20 said peptide consists of the sequence selected from the group consisting of:

GIINANDPLPFFWFMSPYTPGPAPIDINASRALVSNESG (SEQ ID NO: 1),
CGRAYCLSGNYNIFGALFPGVSTPYADVGHDDAQSWRR (SEQ ID NO: 3),
DLSRNLDGFRFLLYNAYVPGFTPTFISLTAEHLSSPKG (SEQ ID NO: 2),
25 RCSPIWGISYPFGLLSSNPGVCHSSDAETNIRNDILTT (SEQ ID NO: 4),

- and
GHSNYCFVSTLGMPIVGFPFSINARGLIHYGGSDPRLAA (SEQ ID NO: 5);
or a binding portion thereof.

- 30 14. A method of identifying a peptide which binds to a substance of interest, comprising:

- (a) screening a first random peptide library
with a ligand, said ligand being a specific
binding partner of said substance of
interest, to identify a first peptide that
specifically binds to said ligand; and
35 (b) screening a second random peptide library
with a compound comprising said first
peptide identified in step (a), to identify
a second peptide which binds to said

or more contiguous sequences, wherein the total number of unpredictable nucleotides is greater than or equal to about 15 and less than or equal to about 600.

5 21. The method of claim 15 in which the library of step (a) or step (b) is a library of recombinant vectors that express a plurality of heterofunctional fusion proteins, said fusion proteins comprising a binding domain encoded by an
10 oligonucleotide comprising unpredictable nucleotides in which the unpredictable nucleotides are arranged in one or more contiguous sequences, wherein the total number of unpredictable nucleotides is greater than or equal to about 15 and less than or equal to about 600.

15 22. The method of claim 14 where the library of step (a) or step (b) is a chemically synthesized library.

20 23. A method of detecting or measuring an analyte of interest in a sample, comprising:

(a) contacting a sample with a molecule comprising a peptide capable of specifically binding said analyte of interest under conditions such that
25 specific binding between said molecule and said analyte can occur; and

(b) detecting or measuring the amount of said binding in which the presence and amount of said binding indicates the presence and amount, respectively, of said analyte in the sample;
30

35 in which said peptide is identified by the method of claim 14.

24. The method of claim 23 in which said molecule is immobilized on a solid substratum.

25. A method of determining the location in a patient of a tumor comprising:

- 5 (a) introducing a molecule comprising a peptide that specifically binds to a tumor antigen into the patient; and
- (b) determining the location in the patient of the molecule;

10 in which the molecule is detectably labeled; and in which said peptide is identified by a method comprising:

- (i) screening a first random peptide library with an antibody or antigen-binding derivative thereof that specifically binds to said tumor antigen, to identify a first peptide that specifically binds to said antibody or antigen-binding derivative thereof; and
- 15 20 (ii) screening a second random peptide library with a molecule comprising said first peptide identified in (i), to identify a second peptide which binds to said molecule and which binds to said tumor antigen.
- 25 30

35 26. A therapeutic or diagnostic composition comprising the molecule of claim 1; and a pharmaceutically acceptable carrier.

27. A therapeutic or diagnostic composition comprising the molecule of claim 2; and a pharmaceutically acceptable carrier.

28. A therapeutic or diagnostic composition comprising the molecule of claim 5; and a pharmaceutically acceptable carrier.

5 29. A therapeutic or diagnostic composition comprising the molecule of claim 7; and a pharmaceutically acceptable carrier.

10 30. A therapeutic or diagnostic composition comprising the molecule of claim 8; and a pharmaceutically acceptable carrier.

15 31. A therapeutic or diagnostic composition comprising the molecule of claim 12; and a pharmaceutically acceptable carrier.

20 32. A composition comprising a plurality of molecules of claim 1, in which said peptide sequences of said molecules differ. -

25 33. A molecule comprising a peptide or a binding portion thereof which binds to a ligand of interest, which peptide is identified by a method comprising: screening a random peptide library with a ligand of interest, said ligand of interest being a peptide having a length of between 5 and 40 amino acids, to identify a peptide that specifically binds to the ligand of interest, in which the ligand of interest is also specifically bound by an antibody or a receptor.

30 34. The molecule of claim 31 in which the ligand is a peptide having a length of between 10 and 20 amino acids.

35 35. A method of obtaining an image of an internal region of a subject comprising administering to said subject an effective amount of the molecule of claim 1 in which said molecule is radiolabeled with a radioactive metal, and recording the scintigraphic image obtained from the decay of said radioactive metal.

36. A molecule comprising a peptide which binds to a substance of interest, which peptide is identified by a method comprising: screening a random peptide library with a ligand, said ligand being a peptide of 36 amino acids or fewer, in which the ligand is an epitope of an antigen that is specifically bound by an antibody or in which the ligand represents the portion of a receptor-ligand that is responsible for the specific binding of the receptor to the receptor-ligand.

10

37. A peptide comprising the amino acid sequence WQGTHF (SEQ ID NO: 23) and the amino acid sequence LVSKND SG (SEQ ID NO: 24) that specifically binds to an antigen of human prostate carcinoma cells.

15

38. A molecule comprising an amino acid sequence selected from the group consisting of:

20 SFMDYFFHTPEPKPAGYPNAYTDPKHFA (SEQ ID NO: 26),
SSSIFDYAFFSWGSAGLSNSSINVFERS (SEQ ID NO: 27),
SASLWDALGGWTTSAVPSYRPHQTPGR (SEQ ID NO: 28),
SLGLPWIDVFGRSSAEFPWFGRTNLPRS (SEQ ID NO: 29),
SVHGAFLDSFFPWAADGPHGRGLTSF (SEQ ID NO: 30),
EEKQGGRWSTMMRPWCHEGGCGFLYYDAMTKPKTPIIMRTAA
(SEQ ID NO: 31),
25 LPRPFDDASWKLRAVKESPDGCGFSGPLLPFYSGLPFTFSSCD
(SEQ ID NO: 32),
GSFESARGVTCIGNHSIGAHGCGPLRSYASFNRGSGRRH (SEQ
ID NO: 33),
DQIGSRPQTTSRSISGSWENAKTLWQQDYAFSAPNAA (SEQ ID
30 NO: 34),
LSDAWGNFTTSYRDSAGFP SHAMTTSQGGKRNHASRFP (SEQ ID
NO: 35),
VQLDDTSPRASGQETSQSEYDARPLL SKFAIRPWSR (SEQ ID
NO: 36),
35 IDSSKNRISGTGYLSFPHIRHANRRHMADDSNLAPGPS (SEQ ID
NO: 37),
WSIGTHTGPEGKFRIPCDRSGCGGTTLTHGGLNSSPTGQHERP
(SEQ ID NO: 38),
DPCEDGYWLLSSVGRAGASIRGCGAIRRSSRTLAEYSTRASNH
(SEQ ID NO: 39),

5 GSKRSCWGTITISNYFRFPVPEGCGSASSINPNTNTGRPLSLHRQ
(SEQ ID NO: 40),
SSASSGCLGRAEHLDLDSVWVGCGSQADMSRRYSPWYGRPRTGV
(SEQ ID NO: 41),
NVMWSSSKAGIRDSCSQVPPGGCGPVNRHRASPLPTPFRHGSIR
(SEQ ID NO: 42),
PLTSGSSSEYRNRDDCPVYKYATNCPRLNFSRSPYSPF (SEQ ID
NO: 43),
GDAYGGIFSRPRQGLADSYIHASYTGKHFFRGPRPPTTR (SEQ ID
NO: 44),
10 STCIGAEGEWKSPHNFLQCRDATSTSSSTLDPTALRFG (SEQ ID
NO: 45),
YSATLWDQFGSRQVELWSNRHASSALPFASRASVLGSR (SEQ ID
NO: 46),
15 ILGWPFPLTGLGDSTVHPRGRKGTDP (SEQ ID NO: 47),
SIPSFMSWLNQLGSAALPSKGNQDRSD (SEQ ID NO: 48),
SRDDIFTGGPLVLFRGSKTSNHDVHSMR (SEQ ID NO: 49),
RAELVNWYEFHWTAEAETPVINSNMT (SEQ ID NO: 50),
GAPVWRGNPRWRGPGGFKWPGCGNGPMCNFTFPARGGSRNNGP
(SEQ ID NO: 51),
20 GSASSCFPNFTARGVTVGFFGCGSPAHPAAPRVLPATDFPAP
(SEQ ID NO: 52),
VFRRTARSSRPIGATVFPWYCGNSNDELPHHDSPPSFFLGA
(SEQ ID NO: 53),
25 NTCWTDLFWHGLPGGDLPRDGCGLPSELTHPSRERRDASEN
(SEQ ID NO: 54),
IDWNWLERGQHNRGYLHSFPDAKSQPTRGRVAPNGND (SEQ ID
NO: 55) and
GRGSDMREHWFPWSMPLILDQHNDSPRAQSHYYSHPF (SEQ ID
NO: 56).

39. A molecule comprising an amino acid sequence selected from the group consisting of:

VSTWGS¹GT²PRWCAPGGKQ³SGSGCGNGPRW⁴TTLPDLGGTRKYGP (SEQ ID NO: 57),
GAPLWCEKLSGTSGSGGFKWP⁵CGSGPT⁶YNTFT⁷PARVGS⁸DNKWP (SEQ ID NO: 58),
GPPVWSAKSRWTGT⁹GVLNWP¹⁰CGCKVPSCST¹¹YTPSRDRSRKSDP (SEQ ID NO: 59),

5 GSALLTSKGCVRGPGGLMRPGCGNDRLGKSSTYAHGGWIKTGP (SEQ ID
 NO: 60),
 GSPVWSGDNRWRGSSPLKRPGCGNGAKCNTLKDNRKDSRKTKH (SEQ ID
 NO: 61),
 10 G PLLPGEAAVHGARGLMRSGCGNGPTWNRLTAACRDSRNKGP (SEQ ID
 NO: 62),
 GSPVWMGSTRWTHGHWFRSQCGGNVPRTNSCAPAGKDSQNKGP (SEQ ID
 NO: 63),
 GAPVWRGNRWCSNDGELERPCCGYGPRFNILPPGRGNSRKPS (SEQ ID
 NO: 64),
 15 GSSGWKVKHRCGGPGTLQRPGCGNPLGHTFPPTRRGGSHMEGA (SEQ ID
 NO: 65),
 GPRSWMGQPRGSDAGSCKWAGCGDAPMWRASTPGHGGPPNRGS (SEQ ID
 NO: 66),
 EALVCRGKPPWSGPAGLLWQCGTGPVSRFTTSAQGRSRNKTS (SEQ ID
 NO: 67),
 GAPVVGDIILWCSGARGAKWPCCGKGPTNKTFSHSRGGTQKSG (SEQ ID
 NO: 68),
 20 GAPVSRCKPACGGFWGVNWPGCGNASMCKTFTNGHGVSSDNH (SEQ ID
 NO: 69),
 GAHGYKNGSTCTGLGGWRCRGCCKGAMCNPSPAGGAYHNQGP (SEQ ID
 NO: 70),
 G PQGSEHQCCSGHWGLKFPCCGNGPICNFTALRGASRNKGP (SEQ ID
 NO: 71),
 25 GEPVWCRHSGGRVQGGDLWLGCGDGPLRYTVTPARGGPKHGP (SEQ ID
 NO: 72),
 GLSLVRGDSWGSGAGGWKRHGCCHGPMYNPQTPARGGSTRNT (SEQ ID
 NO: 73),
 VSRASGKPRLMGSHGLNCPGCGKHSGIMFIPDPAGSANTPP (SEQ ID
 NO: 74),
 30 CAPMWSGKPPWCVGGGVKFRGCGNRPDCNIITPRLVESRDKAL (SEQ ID
 NO: 75) and
 ADPVC SRKPDGGGLRGLRWPGCGKGPILYNVTAARGGSRNNGP (SEQ ID
 NO: 76).
 35

40. The molecule which binds to a ligand of
 interest of claim 33 in which said ligand comprises
 VTSAPDTRPAPGSTAPPAHGVTSAPDTR (SEQ ID NO: 9) or a portion
 thereof.

41. A therapeutic or diagnostic composition comprising a molecule chosen from the group of molecules of claim 38 and a pharmaceutically acceptable carrier.

5 42. A therapeutic or diagnostic composition comprising a molecule chosen from the group of molecules of claim 39 and a pharmaceutically acceptable carrier.

10 43. A molecule that binds to polymorphic epithelial mucin, comprising an amino acid sequence represented by the formula:

$R_1R_2R_3R_4R_5R_6R_7R_8R_9R_{10}R_{11}R_{12}R_{13}R_{14}R_{15}R_{16}R_{17}R_{18}R_{19}R_{20}R_{21}R_{22}R_{23}R_{24}R_{25}R_{26}$
 $R_{27}R_{28}R_{29}R_{30}R_{31}R_{32}R_{33}R_{34}R_{35}R_{36}R_{37}R_{38}R_{39}R_{40}R_{41}R_{42}R_{43}$ (SEQ ID NO: 88)

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wherein:

$R_1 = G, C, E, \text{ or } V;$

$R_2 = A, S, P, \text{ or } L;$

$R_3 = P, T, H, \text{ or } L;$

20 $R_4 = L, M, Q, G, A, \text{ or } S;$

$R_5 = W \text{ or } Y;$

$R_6 = S, C, K \text{ or } T;$

$R_7 = E, S, C, D, V, \text{ or } R;$

$R_8 = N, H, K, S, \text{ or } E;$

25 $R_9 = L, H, R, N, Q, T, \text{ or } G;$

$R_{10} = W, P, R, T, \text{ or } D;$

$R_{11} = W, C, V, L, \text{ or } G;$

$R_{12} = S, T, M, \text{ or } H;$

$R_{13} = G;$

30 $R_{14} = S, A, G, N, Q, \text{ or } H;$

$R_{15} = W, H, G, A, \text{ or } R;$

$R_{16} = G, T, E, P, V, \text{ or } W;$

$R_{17} = V, F, W, K, \text{ or } A;$

$R_{18} = K, Q, D, E, R, \text{ or } L;$

35 $R_{19} = R, F, \text{ or } S;$

$R_{20} = P, S, I \text{ or } H;$

$R_{21} = G;$

$R_{22} = C;$

$R_{23} = G;$

$R_{24} = D, S, T, N;$

- $R_{25} = G, D, L;$
 $R_{26} = P \text{ or } S;$
 $R_{27} = M, S, D, I, L, \text{ or } R;$
 $R_{28} = G, W, C, L, F, Y, \text{ or } T;$
 5 $R_{29} = S, N, V, F, H, \text{ or } R;$
 $R_{30} = N, A, S, M, \text{ or } R;$
 $R_{31} = F, Q, P, \text{ or } V;$
 $R_{32} = S, V, I, K, A, \text{ or } S;$
 $R_{33} = P, A, N, \text{ or } Y;$
 10 $R_{34} = G, N, \text{ or } L;$
 $R_{35} = K, R, C, Q \text{ or } L;$
 $R_{36} = V, K, R, \text{ or } A;$
 $R_{37} = G, D, A, \text{ or } E;$
 $R_{38} = S, T, P, Y \text{ or } W;$
 15 $R_{39} = R, I, L, P, A \text{ or } S;$
 $R_{40} = N, K, \text{ or } M;$
 $R_{41} = S, R, T, E, Q, P, Y \text{ or } H;$
 $R_{42} = G, A, S, D, N, P, Y, \text{ or } K;$
 $R_{43} = P, H \text{ or } A. \quad \sim$

20

44. The molecule of claim 43 wherein:

- $R_1 = G;$
 $R_2 = A;$
 $R_3 = P;$
 25 $R_5 = W;$
 $R_6 = S;$
 $R_{10} = W;$
 $R_{11} = W;$
 $R_{12} = S \text{ or } T;$
 30 $R_{14} = S;$
 $R_{16} = G;$
 $R_{18} = K;$
 $R_{19} = R;$
 $R_{20} = P;$
 35 $R_{26} = P;$
 $R_{28} = G \text{ or } W;$
 $R_{30} = N;$
 $R_{31} = F;$
 $R_{33} = P;$
 $R_{35} = K \text{ or } R;$

R₃₇ = G;

R₃₈ = S;

R₄₀ = N or K;

R₄₂ = G;

5

45. The molecule of claim 2 in which the
antibody or antigen-binding derivative thereof is
capable of specifically binding to a human tumor
antigen.

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ABSTRACT

Abtides are provided. Abtides are peptides identified by a two-step process of screening random peptide libraries. In the first step, the target ligand is an antibody or receptor (or derivative thereof). The peptides identified in the first screening step are used as target ligands in the second screening step. The peptides identified in the second screening step are abtides. Abtides possess binding specificities that are similar to the binding specificities of the antibodies or receptors that are used in the first screening step. Abtides may be used in place of antibodies in many assays or therapeutic applications.

Abtides binding to polymorphic epithelial mucin (PEM) are provided.

Also provided are methods of obtaining abtides as well as diagnostic and therapeutic compounds containing abtides.

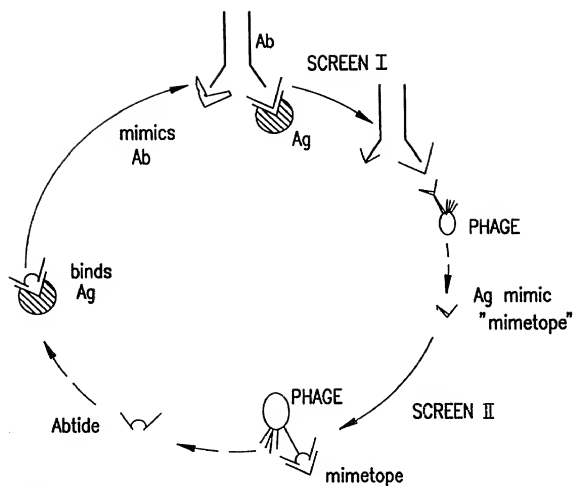


FIG.1

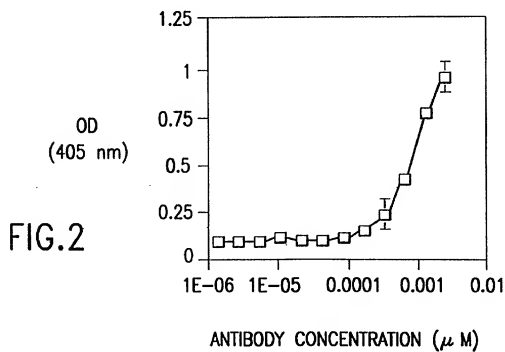


FIG.2

CLONE 14

GIINANDLPFWMS--PYTPGPAPIDINASRALVS-NESG	
WQGFHPYT	LVSKND SG
CDR3L	CDR2L
(5/9 = 55.5 %)	(7/8 = 87.5 %)

CLONE 17

DL-SRNLDFGRFLLYNA--YVPGFTPTFISLTAEHLSSPKG	
LVSKN-DSG	WQGTHTF-P-YT
CDR2L	CDR3L
(6/8 = 75 %)	(6/9 = 66.6 %)

CLONE 15

CGRAYCL-SGNYNIFGALFPGVS--TPYADVGHDDAQSWRR
LVSKN-DS-G WQG-THFPYT
CDR2L CDR3L
(4/8 = 50 %) (6/9 = 66.6 %)

CLONE 13

RCSPIW-GIS-YPFGLLSSNPGVCHSSDAET-NIRNDILT	
WQG-THFPYT	GSDN-K-SVL
CDR3L	CDR2L (REV)
(6/9 = 66.6 %)	(4/8 = 50 %)

CLONE 16

GHSNYCFVSTLGMPIVGFP-SINARGLIHYGGSDPR--LAA
 WQGTHTFPYT GSDNKSVL
 CDR3L CDR2L (REV)
 (3/9 = 33.3 %) (5/8 = 62.5 %)

FIG.3

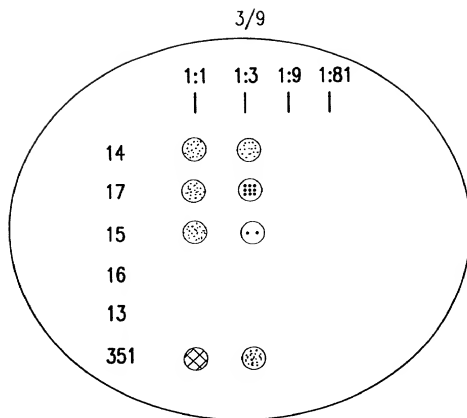


FIG.4

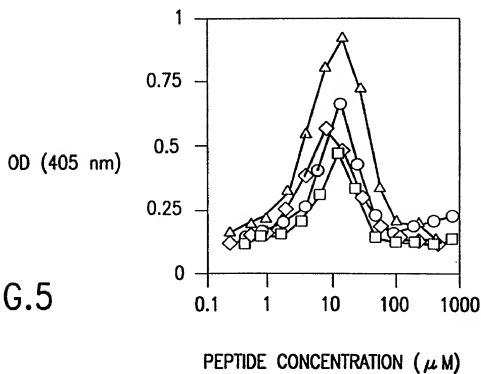


FIG.5

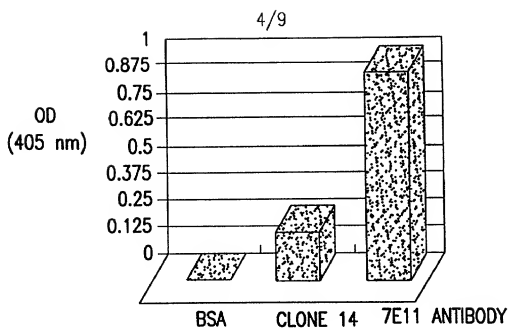


FIG.6

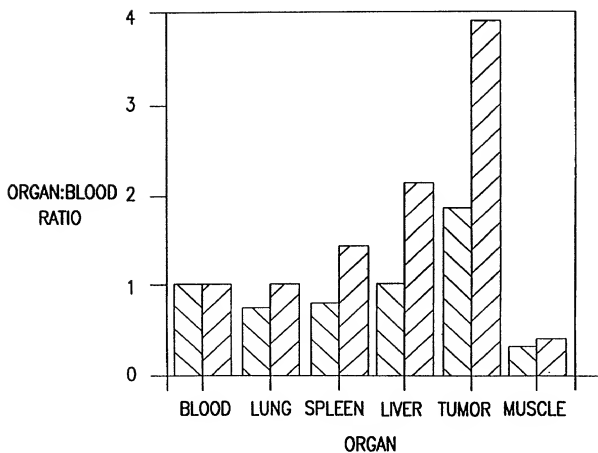
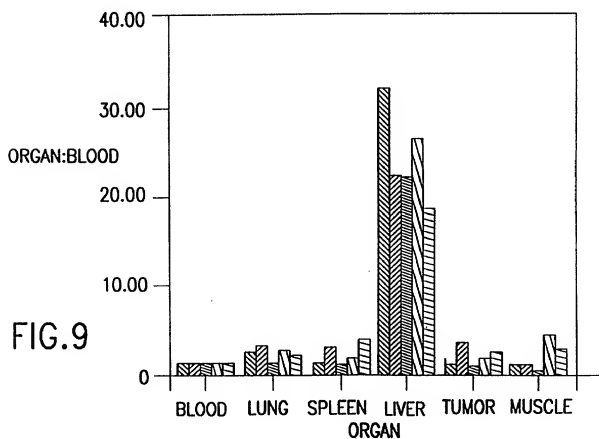
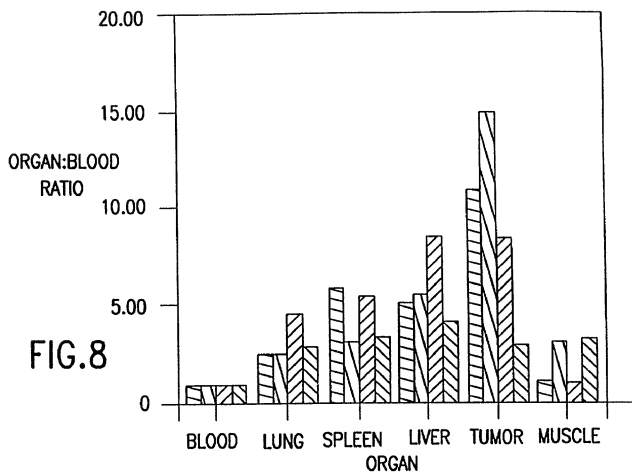


FIG.7



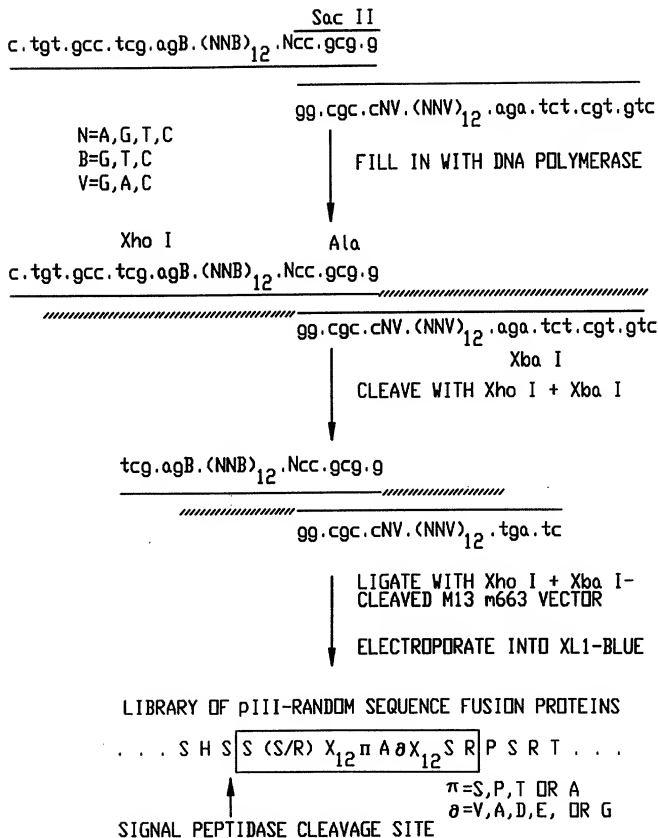


FIG.10

G TGT GTC TCG AGN (NNB)₂₀ NAC GCC AN

N=A,C,G,T
B=C,G,T
V=A,C,G

NTG CGG TNV (NNV)₁₅ AGA TCT GTG TTG

FILL IN WITH SEQUENASE

Xho I

G TGT GTC TCG AGN (NNB)₂₀ NAC GCC AN

NTG CGG TNV (NNV)₁₅ AGA TCT GTG TTG

Xba I

RESTRICT WITH Xho I AND Xba I

TCG AGN (NNB)₂₀ NAC GCC AN

NTG CGG TNV (NNV)₁₅ AGA TC

LIGATE WITH Xho I + Xba I-
CLEAVED M13mp18_{Xa}

ELECTROTRANSFORM
E. coli JS5

D38 GENETIC DIVERSITY LIBRARY DISPLAYED AS RANDOM
N-TERMINAL pIII FUSIONS

. . H S S (S/R) X₂₀ (Y/H/N/D) A (I/M/T/N/K/S/R) X₁₅ S R

↑
SIGNAL PEPTIDASE CLEAVAGE SITE

FIG.11

G TGT GTC TCG AGN (NNB)₂₀ GGT TGT GGT

N=A,C,G,T
B=C,G,T
V=A,C,G

CCA ACA CCA (NNV)₂₀ AGA TCT GTG TTG

FILL IN WITH SEQUENASE

Xho I

G TGT GTC TCG AGN (NNB)₂₀ GGT TGT GGT

CCA ACA CCA (NNV)₂₀ AGA TCT GTG TTG

Xba I

RESTRICT WITH Xho I AND Xba I

TCG AGN (NNB)₂₀ GGT TGT GGT

CCA ACA CCA (NNV)₂₀ AGA TC

LIGATE WITH Xho I + Xba I-
CLEAVED M13mp18Xa

ELECTROTRANSFORM
E. coli JS5

DC43 GENETIC DIVERSITY LIBRARY DISPLAYED AS RANDOM
N-TERMINAL pIII FUSIONS

. . H S S (S/R) X₂₀ G C G X₂₀ S R

↑
SIGNAL PEPTIDASE CLEAVAGE SITE

FIG.12

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below at 201 et seq. underneath my name.

I believe I am the original, first and sole inventor if only one name is listed at 201 below, or an original, first and joint inventor if plural names are listed at 201 et seq. below, of the subject matter which is claimed and for which a patent is sought on the invention entitled

ANTIGEN BINDING PEPTIDES (ABTIDES) FROM PEPTIDE LIBRARIES

the specification of which is attached hereto unless one of the following boxes is checked:

☒ the specification was filed in the United States on June 7, 1995 as Application Serial No. 08/488,161 (for declaration not accompanying application)

with amendment(s) filed on _____ (if applicable)

☐ the specification was filed as PCT international application Serial No. _____ on _____ and was amended under PCT Article 19 on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED PRIOR TO THE FILING DATE OF THE APPLICATION			
APPLICATION NUMBER	COUNTRY	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
			YES <input type="checkbox"/> NO <input type="checkbox"/>
			YES <input type="checkbox"/> NO <input type="checkbox"/>

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

APPLICATION NUMBER	FILING DATE

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS		
		PATENTED	PENDING	ABANDONED
08/310,192	September 21, 1994		X	

POWER OF ATTORNEY: As a named inventor, I hereby appoint S. Leslie Misrock (Reg. No. 18872), Harry C. Jones, III (Reg. No. 20280), Berj A. Terzian (Reg. No. 20060), Gerald J. Flintoft (Reg. No. 20823), David Weild, III (Reg. No. 21094), Jonathan A. Marshall (Reg. No. 24614), Barry D. Rein (Reg. No. 22411), Stanton T. Lawrence, III (Reg. No. 25736), Isaac Jarkovsky (Reg. No. 22713), Joseph V. Colaianni (Reg. No. 20019), Charles E. McKenney (Reg. No. 22795), Philip T. Shannon (Reg. No. 24278), Francis E. Morris (Reg. No. 24615), Charles E. Miller (Reg. No. 24576), Gidon D. Stern (Reg. No. 27469), John J. Lauter, Jr. (Reg. No. 27814), Brian M. Poissant (Reg. No. 28462), Brian D. Coggio (Reg. No. 27624), Rory J. Radding (Reg. No. 28749), Stephen J. Harbulak (Reg. No. 29166), Donald J. Goodell (Reg. No. 19766), James N. Palik (Reg. No. 25510), Thomas E. Friebe (Reg. No. 29258), Laura A. Coruzzi (Reg. No. 30742), Jennifer Gordon (Reg. No. 30753), Jon R. Stark (Reg. No. 30111), Allan A. Fanucci (Reg. No. 30256), Geraldine F. Baldwin (Reg. No. 31232), Victor N. Balancia (Reg. No. 31231), Albert P. Halluin (Reg. No. 25227), and Marcia H. Sundeen (Reg. No. 30893), all of Pennie & Edmonds, whose addresses are 1155 Avenue of the Americas, New York, New York 10036, 1667 K Street N.W., Washington, DC 20006 and 2730 Sand Hill Road, Menlo Park, CA 94025, and each of them, my attorneys, to prosecute this application, and to transact all business in the Patent and Trademark Office connected therewith.

09484879-011800

AND CORRESPONDENCE TO: PENNIE & EDMONDS 1155 AVENUE OF THE AMERICAS NEW YORK, N.Y. 10036-2711				DIRECT TELEPHONE CALLS TO: PENNIE & EDMONDS DOCKETING (212) 790-2803	
2 0 1	FULL NAME OF INVENTOR	LAST NAME Alvarez	FIRST NAME Vernon	MIDDLE NAME L.	
	RESIDENCE & CITIZENSHIP	CITY Morrisville	STATE OR FOREIGN COUNTRY Pennsylvania	COUNTRY OF CITIZENSHIP U.S.A.	
	POST OFFICE ADDRESS	STREET 187 Rice Drive	CITY Morrisville	STATE OR COUNTRY Pennsylvania	ZIP CODE 19067
2 0 2	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	STREET	CITY	STATE OR COUNTRY	ZIP CODE
2 0 3	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	STREET	CITY	STATE OR COUNTRY	ZIP CODE
2 0 4	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	STREET	CITY	STATE OR COUNTRY	ZIP CODE
2 0 5	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	STREET	CITY	STATE OR COUNTRY	ZIP CODE
2 0 6	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME	
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
	POST OFFICE ADDRESS	STREET	CITY	STATE OR COUNTRY	ZIP CODE

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201 VERNON L. ALVAREZ		SIGNATURE OF INVENTOR 202		SIGNATURE OF INVENTOR 203	
DATE 8/9/95		DATE		DATE	
SIGNATURE OF INVENTOR 204		SIGNATURE OF INVENTOR 205		SIGNATURE OF INVENTOR 206	
DATE		DATE		DATE	

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Alvarez, Vernon L.
- (ii) TITLE OF INVENTION: Antigen Binding Peptides (Abtides) From Peptide Libraries
- (iii) NUMBER OF SEQUENCES: 103
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Pennie & Edmonds
 - (B) STREET: 1155 Avenue of the Americas
 - (C) CITY: New York
 - (D) STATE: New York
 - (E) COUNTRY: USA
 - (F) ZIP: 10036
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/488,161
 - (B) FILING DATE: 07-JUN-1995
 - (C) CLASSIFICATION:
- (vii) ATTORNEY/AGENT INFORMATION: ~
 - (A) NAME: Misrock, S. Leslie
 - (B) REGISTRATION NUMBER: 18,872
 - (C) REFERENCE/DOCKET NUMBER: 1101-176
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (212) 790-9090
 - (B) TELEFAX: (212) 869-9741/8864
 - (C) TELEX: 66141 PENNIE

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 38 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Gly	Ile	Ile	Asn	Ala	Asn	Asp	Pro	Leu	Pro	Phe	Trp	Phe	Met	Ser	Pro
1					5				10				15		
Tyr	Thr	Pro	Gly	Pro	Ala	Pro	Ile	Asp	Ile	Asn	Ala	Ser	Arg	Ala	Leu
			20				25						30		
Val	Ser	Asn	Glu	Ser	Gly										
			35												

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 38 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asp Leu Ser Arg Asn Leu Asp Phe Gly Arg Phe Leu Leu Tyr Asn Ala
 1 5 10 15
 Tyr Val Pro Gly Phe Thr Pro Thr Phe Ile Ser Leu Thr Ala Glu His
 20 25 30
 Leu Ser Ser Pro Lys Gly
 35

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 38 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Cys Gly Arg Ala Tyr Cys Leu Ser Gly Asn Tyr Asn Ile Phe Gly Ala
 1 5 10 15
 Leu Phe Pro Gly Val Ser Thr Pro Tyr Ala Asp Val Gly His Asp Asp
 20 25 30
 Ala Gln Ser Trp Arg Arg
 35

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 38 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Arg Cys Ser Pro Ile Trp Gly Ile Ser Tyr Pro Phe Gly Leu Leu Ser
 1 5 10 15
 Ser Asn Pro Gly Val Cys His Ser Ser Asp Ala Glu Thr Asn Ile Arg
 20 25 30

Asn Asp Ile Leu Thr Thr
35

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Gly His Ser Asn Tyr Cys Phe Val Ser Thr Leu Gly Met Pro Ile Val
1 5 10 15

Gly Phe Pro Ser Ile Asn Ala Arg Gly Leu Ile His Tyr Gly Gly Ser
20 25 30

Asp Pro Arg Leu Ala Ala
35

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Trp Gln Gly Thr His Phe Pro Tyr Thr
1 5

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Leu Val Ser Lys Asn Asp Ser Gly
1 5

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Gly Ser Asp Asn Lys Ser Val Leu
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Val Thr Ser Ala Pro Asp Thr Arg Pro Ala Pro Gly Ser Thr Ala Pro
1 5 10 15
Pro Ala His Gly Val Thr Ser Ala Pro Asp Thr Arg
20 25

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Tyr Xaa Xaa Leu His
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ser Cys Val Ser His Met Leu Asp Thr Ser Arg Val Tyr Thr Ala Tyr
1 5 10 15

Ala Asn Pro Gly Met Tyr Ser Arg Leu His Ser Pro Ala Val Arg Pro
20 25 30

Leu Thr Gln Ser Ser Ala
35

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ser Val Gln Phe Lys Ser Ile Ser Ser Arg Ser Met Asp Asp Val Val
1 5 10 15

Lys Asp Pro Gly Pro Lys Pro Ala Met Tyr Asn Arg Leu His Ser Lys
20 25 30

Asn Pro Phe Thr Leu Ser
35

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Tyr Phe Asp His Thr Tyr Ser Gly Pro Val Lys Asn Gly Gly Leu
1 5 10 15

Val Ser Pro Gly Val Leu Ser Met Tyr Asn Arg Leu His Ser Asp Gly
20 25 30

Gly Pro Ser Leu Ala Ser
35

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Thr Val Ala Thr Met His Asp Arg Leu His Ser Ala Pro Gly Ser Gly
1 5 10 15
Asn Leu Pro Gly Ser Tyr Asp Ile Lys Pro Ile Phe Lys Ala Gln Ser
20 25 30
Gly Ala Leu His Ser Thr
35

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Ile Asp Met Pro Gln Thr Ala Ser Thr Met Tyr Asn Met Leu His Arg
1 5 10 15
Asn Glu Pro Gly Gly Arg Lys Leu Ser Pro Pro Ala Asn Asp Met Pro
20 25 30
Pro Ala Leu Leu Lys Arg
35

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Arg Leu Gly Asn His Val Trp Arg Glu Gly Gly Met Tyr Gln Gln
1 5 10 15
Leu His His Asn Phe Pro
20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Arg Asp Ser Ala Val Glu Asn Pro Ser Val Gly Gly Glu Ile Pro Met
1 5 10 15
Tyr Arg Tyr Leu His Gln Arg
20

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Pro Val Gln Lys Glu Tyr Gly Phe Gly Met Ser Gly Ala Ser Met Ile
1 5 10 15
Arg Leu Leu Arg Glu Thr Pro
20

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Gln Lys Gly Gly Pro Gly Leu Leu Leu Tyr Gly Gly Asp Ser Met Tyr
1 5 10 15
Ile Thr Leu His Glu Pro Gly
20

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Leu Tyr Ala Asn Pro Gly Met Tyr Ser Arg Leu His Ser Pro Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gly Met Tyr Ser Arg Leu His Ser Pro Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

His Cys Pro Pro Thr Pro Glu Thr Ser Cys Ala Thr Gln Thr Ile Thr
1 5 10 15

Phe Glu Ser Phe Lys Glu Asn Leu Lys Asp Phe Leu Leu Val Ile Pro
20 25 30

Phe Asp Cys
35

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Arg Glu Pro Val Val Gly Gly Lys Pro Trp Cys Arg Gly Pro Gly Gly
1 5 10 15

Leu Arg Trp Arg Gly Cys Gly Lys Ser Gln Phe Asp Lys Ile Thr
20 25 30

Leu Ser Arg Asp Asn Arg Arg Asp Lys Arg Pro

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 43 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: /label= Xaa

/note= "Xaa = Gly, Cys, Glu, or Val"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2
- (D) OTHER INFORMATION: /label= Xaa

/note= "Xaa = Ala, Ser, Pro or Leu"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3
- (D) OTHER INFORMATION: /label= Xaa

/note= "Xaa = Pro, Thr, His or Leu"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 4
- (D) OTHER INFORMATION: /label= Xaa

/note= "Xaa = Leu, Met, Gln, Gly, Ala, or Ser"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: /label= Xaa

/note= "Xaa = Trp or Tyr"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /label= Xaa

/note= "Xaa = Ser, Cys, Lys or Thr"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 7
- (D) OTHER INFORMATION: /label= Xaa

/note= "Xaa = Glu, Ser, Cys, Asp, Val, or Arg"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 8
- (D) OTHER INFORMATION: /label= Xaa

/note= "Xaa = Asn, His, Lys, Ser or Glu"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 9
- (D) OTHER INFORMATION: /label= Xaa

/note= "Xaa = Leu, His, Arg, Asn, Gln, Thr or Gly"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 10
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Trp, Pro, Arg, Thr, or Asp"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 11
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Trp, Cys, Val, Leu, or Gly"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 12
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Ser, Thr, Met, or His"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 13
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Gly"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 14
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Ser, Ala, Gly, Asn, Gln, or His"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 15
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Trp, His, Gly, Ala, or Arg"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 16
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Gly, Thr, Glu, Pro, Val, or Trp"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 17
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Val, Phe, Trp, Lys, or Ala"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 18
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Lys, Gln, Asp, Glu, Arg, and Leu"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 19
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Arg, Phe, or Ser"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 20
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Pro, Ser, Ile, or His"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
 (B) LOCATION: 21
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Gly"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 22
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Cys"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 23
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Gly"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 24
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Asp, Ser, Thr, or Asn"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 25
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Gly, Asp, or Leu"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 26
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Pro or Ser"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 27
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Met, Ser, Asp, Ile, Leu, or Arg"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 28
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Gly, Trp, Cys, Leu, Phe, Tyr, or Thr"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 29
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Ser, Asn, Val, Phe, His or Arg"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 30
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Asn, Arg, Ser, Met, or Arg"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 31
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Phe, Gln, Pro, or Val"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site

001100-011800

(B) LOCATION: 32
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Ser, Val, Ile, Lys, Ala or Ser"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 33
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Pro, Ala, Asn, or Tyr"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 34
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Gly, Asn, or Leu"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 35
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Lys, Arg, Cys, Gln, or Leu"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 36
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Val, Lys, Arg, or Ala"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 37
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Gly, Asp, Ala, or Glu"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 38
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Ser, Thr, Pro, Tyr, or Trp"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 39
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Arg, Ile, Leu, Pro, Ala, or Ser"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 40
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Asn, Lys, or Met"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 41
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Ser, Arg, Thr, Glu, Gln, Pro, Tyr, or His"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 42
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Gly, Ala, Ser, Asp, Asn, Pro, Tyr, or Lys"

(ix) FEATURE:
 (A) NAME/KEY: Modified-site
 (B) LOCATION: 43

(D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Pro, His, or Ala"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
20 25 30
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
35 40

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 2
(D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Ser or Arg"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 15
(D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Ser, Pro, Thr, or Ala"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 17
(D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Val, Ala, Asp, Glu, or Gly"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
20 25 30 Ser Arg

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Ser Phe Met Asp Tyr Phe Phe His Thr Pro Glu Pro Lys Pro Ala Gly
1 5 10 15

Tyr Pro Asn Ala Tyr Thr Asp Pro Lys His Pro Ala
20 25

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Ser Ser Ser Ile Phe Asp Tyr Ala Pro Phe Ser Trp Gly Ser Ala Gly
1 5 10 15

Leu Ser Asn Ser Ser Ile Asn Val Phe Glu Arg Ser
20 25

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ser Ala Ser Leu Trp Asp Ala Leu Gly Gly Trp Thr Thr Ser Ala Val
1 5 10 15

Pro Ser Tyr Pro Arg Pro His Gln Thr Pro Gly Arg
20 25

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Ser Leu Gly Leu Pro Trp Ile Asp Val Phe Gly Arg Ser Ser Ala Glu
1 5 10 15

Pro Trp Pro Phe Gly Arg Thr Asn Leu Pro Arg Ser

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ser Val His Gly Ala Phe Leu Asp Ser Phe Phe Pro Trp Ala Ala Asp
1 5 10 15
Gly Pro His Gly Arg Gly Arg Leu Thr Ser Phe
20 25

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Glu Glu Lys Gln Gly Gly Arg Trp Ser Thr Met Met Pro Arg Pro Trp
1 5 10 15

Cys His Glu Gly Gly Cys Gly Phe Leu Tyr Tyr Asp Ala Met Thr Lys
20 25 30

Pro Lys Thr Pro Pro Ile Met Arg Thr Ala Ala
35 40

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEO ID NO:32:

Leu Pro Arg Pro Phe Asp Asp Ala Ser Trp Lys Leu Arg Ala Val Lys
1 5 10 15
Glu Ser Pro Asp Gly Cys Gly Phe Gly Ser Pro Leu Leu Phe Pro Pro
20 25 30

1997 **1998** **1999** **2000** **2001** **2002** **2003** **2004** **2005** **2006** **2007** **2008** **2009** **2010** **2011** **2012** **2013** **2014** **2015** **2016** **2017** **2018** **2019** **2020** **2021** **2022** **2023** **2024** **2025** **2026** **2027** **2028** **2029** **2030** **2031** **2032** **2033** **2034** **2035** **2036** **2037** **2038** **2039** **2040** **2041** **2042** **2043** **2044** **2045** **2046** **2047** **2048** **2049** **2050** **2051** **2052** **2053** **2054** **2055** **2056** **2057** **2058** **2059** **2060** **2061** **2062** **2063** **2064** **2065** **2066** **2067** **2068** **2069** **2070** **2071** **2072** **2073** **2074** **2075** **2076** **2077** **2078** **2079** **2080** **2081** **2082** **2083** **2084** **2085** **2086** **2087** **2088** **2089** **2090** **2091** **2092** **2093** **2094** **2095** **2096** **2097** **2098** **2099** **2100** **2101** **2102** **2103** **2104** **2105** **2106** **2107** **2108** **2109** **2110** **2111** **2112** **2113** **2114** **2115** **2116** **2117** **2118** **2119** **2120** **2121** **2122** **2123** **2124** **2125** **2126** **2127** **2128** **2129** **2130** **2131** **2132** **2133** **2134** **2135** **2136** **2137** **2138** **2139** **2140** **2141** **2142** **2143** **2144** **2145** **2146** **2147** **2148** **2149** **2150** **2151** **2152** **2153** **2154** **2155** **2156** **2157** **2158** **2159** **2160** **2161** **2162** **2163** **2164** **2165** **2166** **2167** **2168** **2169** **2170** **2171** **2172** **2173** **2174** **2175** **2176** **2177** **2178** **2179** **2180** **2181** **2182** **2183** **2184** **2185** **2186** **2187** **2188** **2189** **2190** **2191** **2192** **2193** **2194** **2195** **2196** **2197** **2198** **2199** **2200** **2201** **2202** **2203** **2204** **2205** **2206** **2207** **2208** **2209** **2210** **2211** **2212** **2213** **2214** **2215** **2216** **2217** **2218** **2219** **2220** **2221** **2222** **2223** **2224** **2225** **2226** **2227** **2228** **2229** **2230** **2231** **2232** **2233** **2234** **2235** **2236** **2237** **2238** **2239** **2240** **2241** **2242** **2243** **2244** **2245** **2246** **2247** **2248** **2249** **2250** **2251** **2252** **2253** **2254** **2255** **2256** **2257** **2258** **2259** **2260** **2261** **2262** **2263** **2264** **2265** **2266** **2267** **2268** **2269** **2270** **2271** **2272** **2273** **2274** **2275** **2276** **2277** **2278** **2279** **2280** **2281** **2282** **2283** **2284** **2285** **2286** **2287** **2288** **2289** **2290** **2291** **2292** **2293** **2294** **2295** **2296** **2297** **2298** **2299** **2300** **2301** **2302** **2303** **2304** **2305** **2306** **2307** **2308** **2309** **2310** **2311** **2312** **2313** **2314** **2315** **2316** **2317** **2318** **2319** **2320** **2321** **2322** **2323** **2324** **2325** **2326** **2327** **2328** **2329** **2330** **2331** **2332** **2333** **2334** **2335** **2336** **2337** **2338** **2339** **2340** **2341** **2342** **2343** **2344** **2345** **2346** **2347** **2348** **2349** **2350** **2351** **2352** **2353** **2354** **2355** **2356** **2357** **2358** **2359** **2360** **2361** **2362** **2363** **2364** **2365** **2366** **2367** **2368** **2369** **2370** **2371** **2372** **2373** **2374** **2375** **2376** **2377** **2378** **2379** **2380** **2381** **2382** **2383** **2384** **2385** **2386** **2387** **2388** **2389** **2390** **2391** **2392** **2393** **2394** **2395** **2396** **2397** **2398** **2399** **2400** **2401** **2402** **2403** **2404** **2405** **24**

Tyr Ser Gly Leu Pro Thr Phe Ser Ser Cys Asp
35 40

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 39 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Gly Ser Phe Glu Ser Ala Arg Gly Val Thr Cys Ile Gly Asn His Ser
1 5 10 15
Ile Gly Ala His Gly Cys Gly Pro Leu Arg Ser Tyr Ala Ser Phe Asn
20 25 30
Arg Gly Ser Gly Arg Arg His
35

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 38 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Asp Gln Ile Gly Ser Arg Pro Gln Thr Thr Ser Arg Ser Ile Ser Gly
1 5 10 15
Ser Trp Trp Glu Asn Ala Lys Thr Leu Trp Gln Gln Asp Tyr Ala Phe
20 25 30
Ser Ala Pro Asn Ala Ala
35

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 38 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Leu Ser Asp Ala Trp Gly Asn Phe Thr Thr Ser Tyr Arg Asp Ser Ala

0000 **0000** **0000**

Trp Ser Ile Gly Thr His Thr Gly Pro Glu Gly Lys Phe Arg Ile Pro
1 5 10 15

Cys Asp Arg Ser Gly Cys Gly Gly Thr Thr Leu Thr His Gly Gly Leu
20 25 30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 43 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Asp Pro Cys Glu Asp Gly Tyr Trp Leu Ser Ser Val Gly Arg Ala Gly
1 5 10 15

Ala Ser Ile Arg Gly Cys Gly Ala Ile Arg Arg Ser Ser Arg Thr Leu
20 25 30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 43 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

[xi) SEQUENCE DESCRIPTION: SEO ID NO:40:

Gly Ser Lys Arg Ser Cys Trp Gly Thr Thr Ile Ser Asn Tyr Phe Arg
1 5 10 15

Pro Val Pro Glu Gly Cys Gly Ser Ala Ser Ser Ile Asn Pro Asn Thr
 . . 20 25 30

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 43 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TCPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Ser Ser Ala Ser Ser Gly Cys Leu Gly Arg Ala Glu His Leu Asp Leu
1 5 10 15
Asp Ser Val Trp Gly Cys Gly Ser Gln Ala Asp Met Ser Arg Arg Tyr
20 25 30
Ser Pro Trp Tyr Gly Arg Pro Arg Thr Gly Val
35 40

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 43 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Asn Val Met Trp Ser Ser Ser Lys Ala Gly Ile Arg Asp Cys Ser Gln
1 5 10 15
Val Pro Pro Gly Gly Cys Gly Pro Val Asn Arg His Arg Ala Ser Pro
20 25 30
Pro Leu Thr Pro Phe Arg His Gly Ser Ile Arg
35 40

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Pro Leu Thr Ser Gly Ser Ser Ser Glu Tyr Arg Asn Arg Asp Asp Cys
1 5 10 15
Pro Val Tyr Lys Tyr Ala Thr Asn Cys Pro Arg Leu Asn Phe Ser Pro
20 25 30
Ser Arg Tyr Ser Pro Phe
35

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Gly Asp Ala Tyr Gly Gly Ile Phe Ser Arg Pro Arg Gln Gly Leu Ala
 1 5 10 15
 Asp Ser Tyr Ile His Ala Ser Tyr Thr Gly Lys His Phe Phe Arg Gly
 20 25 30
 Pro Arg Pro Pro Thr Arg
 35

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 38 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Ser Thr Cys Ile Gly Ala Glu Gly Glu Trp Lys Ser Phe His Asn Phe
 1 5 10 15
 Leu Gln Cys Arg Asp Ala Thr Ser Thr Ser Ser Ser Thr Leu Asp Pro
 20 25 30
 Thr Ala Leu Arg Phe Gly
 35

(2) INFORMATION FOR SEQ ID NO:46:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 38 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Tyr Ser Ala Thr Leu Trp Asp Gln Phe Gly Ser Arg Gln Val Glu Leu
 1 5 10 15
 Trp Ser Asn Arg His Ala Ser Ser Ala Leu Pro Phe Ala Ser Arg Ala
 20 25 30
 Ser Val Leu Gly Ser Arg

(2) INFORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 26 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Ile	Leu	Gly	Trp	Pro	Phe	Leu	Thr	Gly	Leu	Gly	Asp	Ser	Thr	Val	His
1				5					10					15	
Pro	Arg	Gly	Arg	Lys	Gly	Thr	Asp	Pro	Ser						
			20				25								

(2) INFORMATION FOR SEQ ID NO:48:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 28 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Ser	Ile	Pro	Ser	Phe	Ser	Met	Trp	Leu	Asn	Gln	Leu	Gly	Ser	Ala	Ala
1				5					10					15	
Leu	Pro	Ser	Lys	Gly	Asn	Ser	Gln	Asp	Arg	Ser	Asp				
			20				25								

(2) INFORMATION FOR SEQ ID NO:49:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 28 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Ser	Arg	Asp	Asp	Ile	Phe	Thr	Gly	Gly	Pro	Leu	Val	Leu	Phe	Arg	Gly
1				5					10					15	
Ser	Lys	Thr	Ser	Asn	His	Asp	Val	His	Ser	Met	Arg				
			20				25								

(2) INFORMATION FOR SEQ ID NO:50:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 28 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Arg Ala Glu Leu Val Asn Trp Tyr Glu Trp Phe His Val Thr Ala Glu
 1 5 10 15
 Ala Glu Thr Pro Val Ile Asn Ser His Asn Met Thr
 20 25

(2) INFORMATION FOR SEQ ID NO:51:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 43 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Gly Ala Pro Val Trp Arg Gly Asn Pro Arg Trp Arg Gly Pro Gly Gly
 1 5 10 15
 Phe Lys Trp Pro Gly Cys Gly Asn Gly Pro Met Cys Asn Thr Phe Thr
 20 25 30
 Pro Ala Arg Gly Gly Ser Arg Asn Asn Gly Pro
 35 40

(2) INFORMATION FOR SEQ ID NO:52:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 43 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Gly Ser Ala Ser Ser Cys Phe Pro Asn Phe Thr Ala Arg Gly Val Thr
 1 5 10 15
 Val Gly Phe Phe Gly Cys Gly Ser Pro Ala His Pro Ala Ala Pro Arg
 20 25 30
 Val Leu Asn Pro Ala Thr Asp Phe Pro Ala Pro
 35 40

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TO: JCRC
INFO: JCRC
SUBJ: JCRC
PRIORITY: 030813Z 110600Z

(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Val Phe Arg Arg Thr Ala Arg Ser Ser Arg Pro Ile Gly Ala Thr Val
1 5 10 15

Phe Pro Trp Tyr Gly Cys Gly Asn Ser Asn Asp Glu Thr Leu Pro His
20 25 30

His Asp Ser Pro Pro Ser Phe Phe Leu Gly Ala
35 40

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Asn Thr Cys Trp Thr Asp Leu Phe Trp His Gly Leu Pro Gly Gly Asp
 1 5 10 15
 Leu Pro Arg Asp Gly Cys Gly Leu Pro Ser Glu Leu Thr Thr His Pro
 20 25 30
 Ser Arg Glu Arg Arg Asp Ala Ser Glu Asn
 35 40

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Ile Asp Trp Asn Trp Leu Glu Arg Gly Gln His Asn Arg Gly Tyr Leu
1 5 10 15
His Ser Phe Pro Asp Ala Lys Ser Gln Pro Thr Arg Gly Pro Arg Val

Ala Pro Asn Gly Asn Asp
35

(2) INFORMATION FOR SEQ ID NO:56:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 38 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Gly Arg Gly Ser Asp Met Arg Glu His Trp Pro Trp Ser Met Pro Leu
1 5 10 15

Ile Leu Asp Gln His Ala Asn Asp Pro Ser Pro Arg Ala Gln Ser His
20 25 30

Tyr Tyr Ser His Pro Phe
35

(2) INFORMATION FOR SEQ ID NO:57:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 43 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Val Ser Thr Gly Trp Ser Gly Thr Pro Arg Trp Cys Ala Pro Gly Gly
1 5 10 15

Lys Gln Gly Ser Gly Cys Gly Asn Gly Pro Arg Trp Thr Thr Leu Thr
20 25 30

Pro Asp Leu Gly Gly Thr Arg Lys Tyr Gly Pro
35 40

(2) INFORMATION FOR SEQ ID NO:58:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 43 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Gly Ser Pro Val Trp Ser Gly Asp Asn Arg Trp Arg Gly Ser Ser Pro
1 5 10 15
Leu Lys Arg Pro Gly Cys Gly Asn Gly Ala Lys Cys Asn Thr Leu Lys
20 25 30
Asp Asn Arg Lys Asp Ser Arg Lys Thr Lys His
35 40

(2) INFORMATION FOR SEQ ID NO:62:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Gly Pro Leu Leu Pro Gly Glu Ala Ala Val His Gly Ala Arg Gly Leu
1 5 10 15
Met Arg Ser Gly Cys Gly Asn Gly Pro Thr Trp Asn Arg Leu Thr Ala
20 25 30
Ala Cys Arg Asp Ser Arg Asn Lys Gly Pro
35 40

(2) INFORMATION FOR SEQ ID NO:63:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Gly Ser Pro Val Trp Met Gly Ser Thr Arg Trp Thr Gly His Gly Trp
1 5 10 15
Phe Arg Ser Gln Gly Cys Gly Asn Val Pro Arg Thr Asn Ser Cys Ala
20 25 30
Pro Ala Gly Lys Asp Ser Gln Asn Lys Gly Pro
35 40

(2) INFORMATION FOR SEQ ID NO:64:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

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- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 43 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Glu Ala Leu Val Cys Arg Gly Lys Pro Pro Trp Ser Gly Pro Ala Gly
 1 5 10 15
 Leu Leu Trp Gln Gly Cys Gly Thr Gly Pro Val Ser Arg Thr Phe Thr
 20 25 30
 Ser Ala Gln Gly Arg Ser Arg Asn Lys Thr Ser
 35 40

(2) INFORMATION FOR SEQ ID NO:68:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 43 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Gly Ala Pro Val Val Gly Asp Ile Leu Trp Cys Ser Gly Ala Arg Gly
 1 5 10 15
 Ala Lys Trp Pro Gly Cys Gly Lys Gly Pro Thr Asn Lys Thr Phe Ser
 20 25 30
 His Ser Arg Gly Gly Thr Gln Lys Ser Gly Leu
 35 40

(2) INFORMATION FOR SEQ ID NO:69:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 43 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

Gly Ala Pro Val Ser Arg Cys Lys Pro Ala Cys Gly Gly Phe Trp Gly
 1 5 10 15
 Val Asn Trp Pro Gly Cys Gly Asn Ala Ser Met Cys Lys Thr Phe Thr
 20 25 30

[illegible]

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 43 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

(2) INFORMATION FOR SEQ ID NO:71:

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Gly Glu Pro Val Trp Cys Arg His Ser Gly Gly Arg Val Gln Gly Gly

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

Cys Ala Pro Met Trp Ser Gly Lys Pro Pro Trp Cys Val Gly Gly Gly
1 5 10 15
Val Lys Phe Arg Gly Cys Gly Asn Arg Pro Asp Cys Asn Ile Ile Thr
20 25 30
Pro Arg Leu Val Glu Ser Arg Asp Lys Ala Leu
35 40

(2) INFORMATION FOR SEQ ID NO:76:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

Ala Asp Pro Val Cys Ser Arg Lys Pro Asp Gly Gly Gly Leu Arg Gly
1 5 10 15
Leu Arg Trp Pro Gly Cys Gly Lys Gly Pro Ile Leu Tyr Asn Val Thr
20 25 30
Ala Ala Arg Gly Gly Ser Arg Asn Asn Gly Pro
35 40

(2) INFORMATION FOR SEQ ID NO:77:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

Gly Thr Arg Val Pro Pro Gly Phe Ala Leu Arg Gly Gly Arg Asp Gly
1 5 10 15
Leu Ser Trp Ala Gly Cys Gly Lys Ala Pro Ile Ser Lys Thr Tyr Thr
20 25 30
Ser Ala Arg Gly Arg Ser Arg Lys Lys Gly Ser
35 40

(2) INFORMATION FOR SEQ ID NO:78:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Arg Ser Ala Val Ser Glu Gly Lys Pro Arg Glu Ile Val Pro Gly Gly
1 5 10 15
Cys Met Trp Pro Gly Cys Gly Asn Gly Arg Lys Ser Asn Thr Leu Thr
20 25 30
His Gly Pro Glu Gln Phe Gln Glu Ile Glu Pro
35 40

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

Ser Ser Gly Val Gly Asn Gly Lys Pro Arg Ser Trp Ala Pro Asp Ala
1 5 10 15
Leu Asn Gly Gly Cys Gly Asn Ile Gln Phe Ala Asn Thr Ile Thr Pro
20 25 30
Asp Arg Gly Gly Ser Cys Asn Gln Thr Leu
35 40

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 43 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

Gly Ser Ser Val Cys Gly Gly Gln Pro Ser Gly Arg Gly Phe Gly Gly
1 5 10 15
Leu Pro Gly Pro Gly Cys Gly Asn Gly Pro Thr Ser Asn Thr Leu Thr
20 25 30
Ser Ala Arg Gly Gly Phe Pro Asn Lys Gly Leu
35 40

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 43 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Gly Ala Pro Leu Trp Gln Gly Asp Pro Ala Asp Glu Val Leu Gly Gly
 1 5 10 15
 Ser Met Ile Pro Gly Cys Gly Ile Gly Ala Leu Ser Gln Thr Phe Thr
 20 25 30
 Pro Thr Pro Gly Gly Ser Arg Lys Asn Val Thr
 35 40

(2) INFORMATION FOR SEQ ID NO:82:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 42 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

Ala Gly Arg Glu Leu Arg Gln Asp Glu Gly Glu Gly Gly Ala Gly Ala
 1 5 10 15
 Asp Val Ala Arg Leu Arg Glu Gly Pro Ile Cys Ser Thr Phe Thr Pro
 20 25 30
 Ala Arg Gly Gly Ser Cys Pro Ser Gly Leu
 35 40

(2) INFORMATION FOR SEQ ID NO:83:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 43 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

Gln Ala Arg Val Ser Met Ala Ile Ser Cys Arg Ser Gly Pro Ser Asp
 1 5 10 15
 Leu Met His Gln Gly Cys Gly Tyr Gly Pro Arg Cys Asn Pro Asp Thr
 20 25 30
 Thr Asp Ser Gly Gly Ser His Thr Asn Thr Pro

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

Gly Asp Pro Glu Cys Arg Gly Lys Pro Arg Gly Arg Trp Thr Gly Ser
1 5 10 15

Leu Ala Cys Thr Gly Cys Gly Asn Gly Pro Asn Ser Lys Ile Cys Thr
20 25 30

Arg Ala Arg Gly Val Ser Arg Asn Lys Gly Pro
35 40

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 43 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Ser Thr Pro Gly Cys Ser Gly Tyr Ser Gly Ser Gly Asp Pro Arg Cys
1 5 10 15

Leu Thr Cys Thr Ala Cys Gly Asn Gly His Thr Arg Lys Thr Leu Thr
20 25 30

Pro Ala His Gly Arg Ser Thr His Lys Glu Pro
35 40

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Gly Gln Pro Glu Cys Arg Ile Thr Ser Gly Cys Cys Gly Thr Asp Gly
1 5 10 15

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Asn Lys Trp Leu Gly Cys Gly Lys Val Asp Met Cys Asn Thr Leu Asn
20 25 30

Pro Ala Val Gly Cys His Gly Thr Asn Gly Ser
35 40

(2) INFORMATION FOR SEQ ID NO:87:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 56 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

CTGTGCCTCG AGBNBNNNBN NBNBNNBNN BNNBNNBNNB NNBNNBNNBN CGCGCG

56

(2) INFORMATION FOR SEQ ID NO:88:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 56 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

CTGTGCTCTA GAVNNVNNVN NVNNVNNVNN VNNVNNVNV NVNNVNNVN CCGCGG

56

(2) INFORMATION FOR SEQ ID NO:89:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 49 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

TCGAGBNNBN NBNBNNBNN BNNBNNBNNB NNBNNBNNBN NBNCCGCGG

49

(2) INFORMATION FOR SEQ ID NO:90:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 49 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

CTAGTVNNVN NVNNVNNVNN VNNVNNVNNV NNVNNVNNVN NVNCCGCGG

49

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: /label= Xaa

/note= "Xaa = Ser or Arg"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 18
- (D) OTHER INFORMATION: /label= Xaa

/note= "Xaa = Ser, Pro, Thr, or Ala"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 20
- (D) OTHER INFORMATION: /label= Xaa

/note= "Xaa = Val, Ala, Asp, Glu, or Gly"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

Ser His Ser Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Ala Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
20 25 30

Ser Arg Pro Ser Arg Thr
35

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 81 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

GTGTGTCGTCG AGNNNBNNBN NBNNBNBNBN BNNBNBNBNB NNBNNBNBNB NBNNBNBNBN

60

BNNBNBNBNB NNBACGCCA N

81

(2) INFORMATION FOR SEQ ID NO:93:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

GTGTGTCTA GAVNNVNNVN NVNNVNNVNN VNNVNNVNNV NNVNNVNNVN NVNNVNNVNT 60
GGCGTN 66

(2) INFORMATION FOR SEQ ID NO:94:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 74 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

TCGAGNNNBN NBNBNNBNN BNNBNNBNNB NBNBNNBNNB NBNBNNBNN BNNBNNBNNB 60
NNBNNBNACG CCAN 74

(2) INFORMATION FOR SEQ ID NO:95:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

CTAGAVNNVN NVNNVNNVNN VNNVNNVNNV NNVNNVNNVN NVNNVNNVNN VNTGCCGTN 59

(2) INFORMATION FOR SEQ ID NO:96:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 4

(D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Ser or Arg"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 25
(D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Tyr, His, Asn or Asp"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION: 27
(D) OTHER INFORMATION: /label= Xaa
/note= "Xaa = Ile, Met, Thr, Asn, Lys, Ser, or Arg"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

```
His Ser Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1          5          10          15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ala Xaa Xaa Xaa Xaa Xaa Xaa
20          25          30
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ser Arg
35          40
```

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 82 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

```
GTGTGTCCTCG AGNNBNBNNBN NBNNBNBNNBN BNNBNBNNBNB NBNBNBNNBN NBNNBNBNNBN      60
BNNBNBNNBNB NNBGGTTGTG GT                                                    82
```

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

```
GTGTGTCCTA GAVNNVNNVN NVNNVNNVNN VNNVNNVNNV NVNNVNNVNN NVNNVNNVNN      60
VNNVNNVNNV NNACCACAC C                                                         81
```

(2) INFORMATION FOR SEQ ID NO:99:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 75 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

TCGAGNNBNB NBNBNBNBNB BNNBNBNBNB NBNBNBNBNB NBNBNBNBNB BNNBNBNBNB 60
 NBNBNBGGTT GTGGT 75

(2) INFORMATION FOR SEQ ID NO:100:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 74 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

CTAGAVNNVN NVNNVNNVNN VNNVNNVNN NVNNVNNVN NVNNVNNVN VNNVNNVNNV 60
 NNVNACCAC AACC 74

(2) INFORMATION FOR SEQ ID NO:101:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 49 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Modified-site
 (B) LOCATION: 4
 (D) OTHER INFORMATION: /label= Xaa
 /note= "Xaa = Ser or Arg"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

His Ser Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 1 5 10 15
 Xaa Xaa Xaa' Xaa Xaa Xaa Xaa Gly Cys Gly Xaa Xaa Xaa Xaa Xaa
 20 25 30
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ser
 35 40 45

Arg

(2) INFORMATION FOR SEQ ID NO:102:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 69 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

GGSGCSCCSG TSTGSAGSGG SAASCCSCGS TGSAGSGGSC CSGGSGGTTT SAASTGSCCS	60
GGCTGCGGG	69

(2) INFORMATION FOR SEQ ID NO:103:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 69 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

SGGSCCSTTS TTSCG3GASC CSCCSCGSGC SGGSGTSAAS GTSTTSCASA TSGGSCCSTT	60
CCCGCAGCC	69